TRANSFUSION

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Editorial

Blood Banking and the Challenge of Extracorporeal Circulation

DURING the past five years the rapid developments in surgery employing extracorporeal circulation have been widely adopted. Thus the multiple problems these advances have brought to blood banks are no longer confined only to the large medical centers. Unfortunately many of these problems, ranging from procurement of large amounts of type-specific fresh blood to special anticoagulant preservative mixtures, remain unsolved. In addition, new problems for blood banks have recently emerged now that extracorporeal circulation has been combined with "perfusion thermoregulation" for inducing varying depths of hypothermia.

In 1959 Smith, *et al.*,[5] reported the experience at Duke University with a new anticoagulant preservative, Edglugate-Mg. This solution was especially designed for extracorporeal circulation so as to permit a short storage period (up to five days) for donor blood before its use for this purpose. While this anticoagulant preservative mixture has proved helpful and continues to be used in some centers, it is still not ideal. Perhaps its most serious limitation relates to those centers using pump oxygenators that require large priming volumes of donor blood. Since the Edglugate formula contains disodium EDTA which cannot be metabolized and must be excreted entirely by the kidneys, there is a limit to the amount of this blood that can be safely administered during a perfusion.

ACD blood with its many advantages for routine transfusion has in the past been considered unsuitable for perfusion because of its additional acid, sodium, and water content and the large and uncertain amount of calcium ion required to restore a proper ionized calcium level after its heparinization. Yet, at one time or another most surgical teams have had to add to their pump reservoirs some banked ACD blood when in the course of perfusions their supply of fresh heparinized blood became exhausted. In most instances this has caused no difficulties and has led to renewed interest in the possible use of ACD blood for the total pump priming volume. In the past year, at least one pump oxygenator of moderately small volume has been introduced that is primed retrograde from the artery cannulated for the pump inflow while the patient is transfused intravenously with an equivalent amount of stored ACD blood to maintain a normal intracorporeal volume. More recently Foote, *et al.*,[2] have presented evidence that ACD blood (heparinized then recalcified just before use) may be just as effective for extracorporeal circulation as fresh heparinized blood. However, before this question can be settled much more experimental data and clinical experience must be evaluated. Furthermore, it would seem logical that if citrate becomes acceptable as the initial anticoagulant for such donor bloods, some modification of the present ACD formula will still be necessary. Perhaps another approach to this problem would be to add heparin to stored citrated blood then allow it to flow through a small compact dialyzer during the priming of the heart lung machine. Anthone, *et al.*,[1] have shown that ammonia, potassium and citrate levels can be markedly reduced and the ionized calcium restored to near normal when stored citrated blood is allowed to flow in one pass through a small efficient dialyzer at flow rates up to 120 cc. per minute.

Combining hypothermia, particularly deep hypothermia, with extracorporeal circulation for certain cardiac and neurosurgical procedures raises the question of additional compatibility tests between donor's and patient's blood. For one reason or another and at various times many individuals may exhibit cold agglutinins in their blood that are active at temperatures above those now employed during deep hypothermic perfusion. While little is known concerning the real importance of cold agglutinins in deep hypothermia, all available evidence indicates that at low tissue temperatures they cause intravascular red cell clumping with capillary and venule obstruction. Thus when deep hypothermic perfusion is contemplated, one set of compatibility tests should be carried out at 5 C. so as to eliminate donor bloods with significant cold agglutinin content. When cold agglutinins are found in a patient scheduled for cold perfusion, the surgeon should be notified of their thermal amplitude in order that the patient's temperature be maintained above this level during operation. Should the surgeon still wish to lower the patient's temperature below this level, perhaps the question should be raised of delaying the operation until the patient's cold agglutinins disappear. Another alternative, yet unexplored, might be pre-operative exchange transfusion to lower the patient's cold agglutinin titer. One perfusion team[4] recommends adding low molecular weight dextran to the priming blood volume to decrease blood sludging and resulting capillary stasis that occurs even during normothermic perfusion. Whether this would have an inhibitory effect against cold agglutination is not known at present. A further listing of problems raised by the recent addition of hypothermia to perfusion technics might include the observation of Thomas, *et al.*,[6] that some human bloods anticoagulated with heparin develop a protein precipitate on cooling. Conceivably such a precipitate might cause serious trouble during deep hypothermic perfusion but, as with many of these problems, knowledge is completely lacking on this point.

Lee, *et al.*,[3] in a recent presentation called attention to a number of changes occurring in the blood during extracorporeal circulation which may injure the patient. Undoubtedly the prevention of most of these will require the development of improved artificial heart lung circuits, particularly better oxygenators. Nevertheless, the selection of blood donors, limits of the storage time, the use of certain additives, preservatives and anticoagulants, and perhaps special pre-operative blood testing will certainly play an equally important role.

Perfusion with an extracorporeal pump oxygenator has already taken a permanent place among surgical procedures and all these and the many other blood problems of extracorporeal circulation present a real challenge in a new research area for blood banks.—IVAN W. BROWN, JR., M.D.

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Studies on Bank Blood Collected and Stored under Various Conditions with Particular Reference to Its Use in Open Heart Surgery*

HERBERT A. PERKINS, M.D., MARY R. ROLFS, B.S., D. J. ACRA

From the Research Laboratory of the Irwin Memorial Blood Bank of the San Francisco Medical Society, San Francisco, California

A few of the factors responsible for the preservation of the characteristics of fresh blood during storage have been studied, with particular reference to the provision of donor blood for open heart surgery. It has been indicated that present data leave us no reason to think that platelet viability and the level of labile clotting factors in stored blood will be any different whether the blood is collected by gravity or with the aid of vacuum. Changes in bloods stored at 4 C. for a 21 day period were investigated with ACD, heparin and Edglugate-Mg as the anticoagulants. The studies on heparinized blood suggest that after two or three days, the fall in platelets and the rise in plasma potassium and hemoglobin are so much greater than occur in ACD that storage for longer periods than that will probably never be safe. On the other hand, *in vivo* studies indicate that 24-hour-old heparinized blood is just as safe to use as four-hour-old blood. Edglugate-Mg was developed in an effort to get around the limited storage period of heparinized blood. Results reported here show that plasma potassium levels rise very rapidly in this type of blood. Platelets are not well-preserved *in vitro*, and Factor V levels are apparently low soon after collection.

IN MOST CASES when blood is transfused the chief goal is the addition of viable red cells to the recipient's circulation. Changes in other components in the donor blood induced by collection and storage are of minor importance. This situation is being altered, however, with the increasingly common practice of transfusing massive amounts of blood (due to the development of more vigorous approaches to the massively bleeding patient, the employment of radical surgical technics which are inevitably associated with losses of large amounts of blood, and the increasingly widespread use of artificial heart lung machines in open heart surgery). Under these circumstances all of the altered characteristics of the donor blood resulting from collection and storage become of very great importance, inasmuch as the end result is replacement of most of the recipient's circulating volume with donor blood.

The alterations that occur with the collection and storage of bank blood are multiple. Krevans[13] has shown that massive transfusion of blood with non-viable platelets can result in a bleeding state due to thrombocytopenia. Other coagulation factors which disappear from bank blood during storage have also been implicated. Donor blood is prevented from clotting by binding calcium with a citrate solution. Massive transfusion of citrated blood may conceivably result in lowering the concentration of ionic calcium in the recipient's plasma to a level that can result in cardiac irregularity or standstill.[7] Moreover, the rise in plasma potassium that occurs in bank blood during storage (see below) can have the same effect.

During the course of open heart surgery, the donor blood used must be prevented from clotting by an anticoagulant which does not bind calcium, since the patient's plasma level of ionic calcium must be kept within a normal range. Heparin is the only suitable agent. Blood collected in heparin

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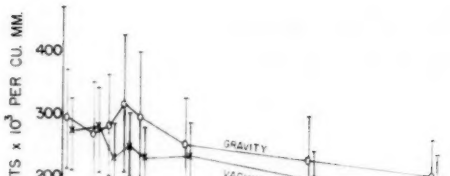

FIG. 1. Changes in platelet counts on storage of ACD blood. A comparison of the effects of vacuum *vs.* gravity collection.

undergoes the changes induced by storage much faster than that collected in the standard acid-citrate-dextrose (ACD) solutions.

It has thus become essential to have definite information on the rate at which blood loses its desirable characteristics and gains undesirable ones under various conditions of collection and storage. There is great need for a technic which will permit retention of all the characteristics of fresh blood during storage for a sufficient period to eliminate the requirement to call in and bleed type-specific donors each time fresh blood is desired. Ideally, such blood should be available not only for routine transfusions but should be adaptable to use in open heart surgery.

With these thoughts in mind, the changes that occur in blood collected and stored in several different anticoagulant solutions have been explored. The effect of gravity *vs.* vacuum collection on ACD blood has been investigated. Studies on heparinized blood have been carried out during *in vitro* storage for a period of 21 days. A separate investigation was concerned with the comparative effect *in patients* of four and 24 hour old heparinized blood. Finally, *in vitro* changes have been followed in a series of bloods collected in Edglugate-Mg, an anticoagulant proposed for use in storing blood before its employment in open heart surgery.

Materials and Methods

Methods of Blood Collection: All blood for this study was collected by the procurement staff of the Irwin Memorial Blood Bank of the San Francisco Medical Society under conditions which ensured that routine phlebotomy procedures (without special precautions) would be used. Each series tested for *in vitro* changes consisted of 12 units of blood.

Four hundred and eighty ml. of blood were collected in 120 ml. of ACD solution (NIH Formula B) in siliconed bottles.** In the series collected by gravity technic the bottles were in an upright position with an air inlet needle in the cap. Disposable plastic donor tubing was employed throughout.*** As the blood flowed in, the bottles were occasionally mixed with a gentle swirl. The same type of bottle was used for the vacuum series. Vacuum bottles were held in an inverted position and the rate of flow into the bottle was controlled by a clamp to prevent a high fountain of blood splashing into the bottle.

Heparinized blood for the *in vivo* studies, which were performed several years ago,

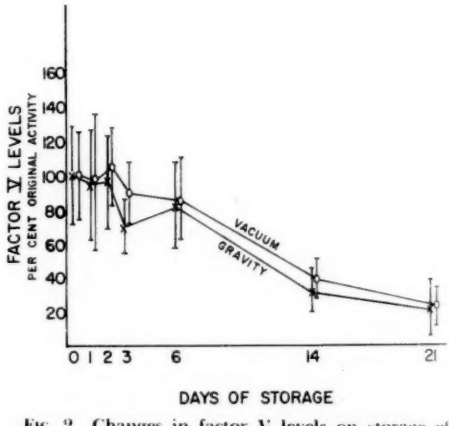

FIG. 2. Changes in factor V levels on storage of ACD blood. A comparison of the effects of vacuum *vs.* gravity collection.

was collected by gravity in empty plastic bags*** to which had been added 2,500 units of heparin sodium† in 2.5 ml. normal saline. Heparinized blood for the more recent *in vitro* studies was made available to us 24 hours after collection by last minute cancellation of an operation. In this series 500 ml. of blood were collected in commercially available heparin bags containing 2,000 units of heparin sodium in 50 ml. buffered saline.†† Collection was assisted by a vacuum device.†††

Five hundred ml. of blood were collected in Edglugate-Mg solution using plastic bags containing 30 ml. of the anticoagulant. Collection was assisted by the same vacuum device mentioned above. These bags were obtained from the manufacturer who made them available on an experimental basis. The anticoagulant solution was devised by Brown and his co-workers at Duke University.[27] It contained the minimum amount of disodium ethylenediamine tetracetic acid (EDTA) believed necessary to prevent blood coagulation consistent with safety. Sodium gluconate was added because of the demonstration that it lessened spontaneous hemolysis of the red cells. Magnesium ion was added because of its apparent effect in minimizing loss of activity of certain coagulation factors. Glucose was in the formula to aid in the preservation of red cell viability.

** Courtland Laboratories, Los Angeles, California.

*** Cutter Laboratories, Berkeley, California.

† Heparin Sodium, Upjohn, 1,000 U per ml.

†† Fenwal Laboratories, Framingham, Massachusetts.

††† Hemolator, Fenwal Laboratories, Framingham, Massachusetts.

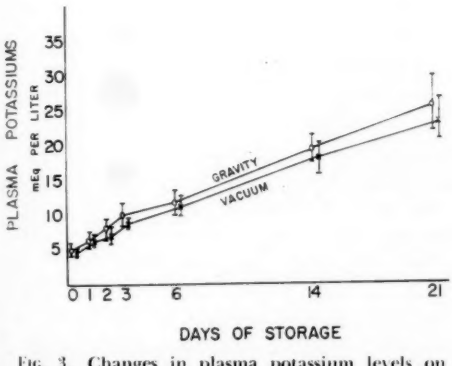

FIG. 3. Changes in plasma potassium levels on storage of ACD blood. A comparison of the effects of vacuum *vs.* gravity collection.

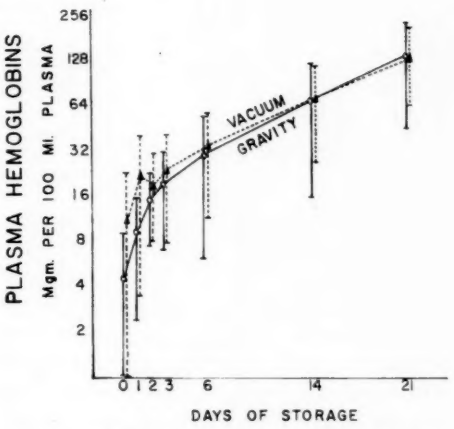

FIG. 4. Changes in plasma hemoglobin levels on storage of ACD blood. A comparison of the effects of vacuum *vs.* gravity collection. (Note change to log scale on vertical axis of this figure.)

In all cases where control platelet counts were done, the sample used was obtained by collecting 5 ml. of donor blood in a 15 ml. polyethylene vial containing 0.2 ml. of 10 per cent EDTA. The vial was filled from the end of the donor tubing as soon as the bottle or bag had been filled. Control studies for plasma coagulation factors were done on a similarly obtained sample of blood, 9 ml. of blood being mixed with 1 ml. of 3.8 per cent sodium citrate in a siliconed glass tube.

Methods of Obtaining Samples for Testing: In the studies of *in vitro* changes on storage all bottles or bags were inverted gently 100 times before sampling. Aliquots were withdrawn with the aid of a siliconed syringe using aseptic technic. Samples were withdrawn from the bags in such a manner

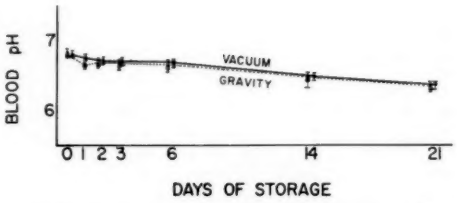

FIG. 5. Changes in blood *p*H on storage of ACD blood. A comparison of the effects of vacuum *vs.* gravity collection.

that additional air was not permitted to enter. Between samplings the containers remained at 4 C. The repeated shaking of the containers might have increased the changes in the bloods as they were stored, but this is a fair test since bank blood is frequently shaken by being transferred from one institution to another before use.

In the *in vivo* studies, samples of venous blood for platelet counts were taken from the patients with siliconed syringes, using a two-syringe technic. Five ml. of blood were added to 0.2 ml. 10 per cent EDTA in a plastic vial whether or not the blood already contained heparin. Donor blood was sampled after the eight donor units had been mixed by recirculation in the pump-oxygenator before connection to the patient. These *in vivo* studies were done with the cooperation of Doctors Frank Gerbode and John Osborn at the Stanford University School of Medicine. The pump-oxygenator used was one of their earlier models, which employed roller pumps and plastic bag filming oxygenators.[20] Because of variable dilution with saline in the machine, all platelet counts on donor blood were corrected by assuming that the original hematocrit of the mixed donor blood had been 43 per cent. The entire series of bypasses was done within a short period by the same surgical team using the same technics of bypass. The volume of donor blood used and the average weights of patients and durations of bypass were the same in the two series compared.

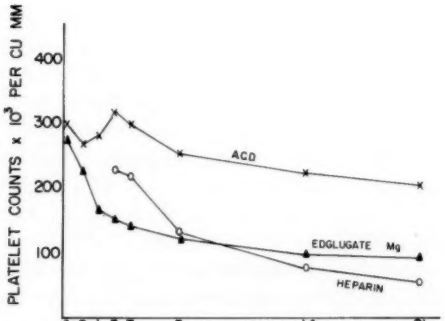

FIG. 6. A comparison of changes in platelet counts on storage of blood in ACD, heparin and Edglugate-Mg.

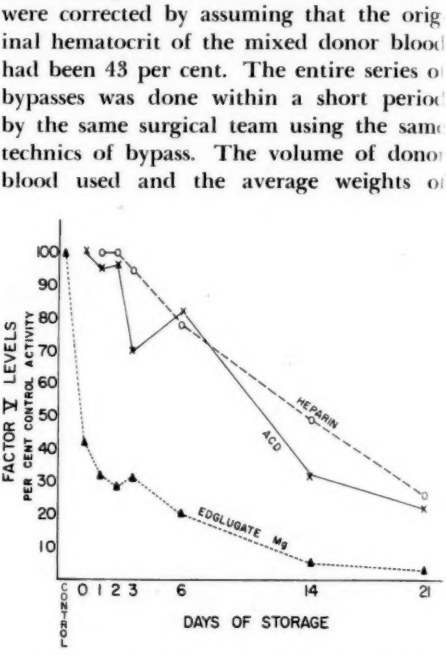

FIG. 7. A comparison of changes in factor V levels on storage of blood in ACD, heparin and Edglugate-Mg.

Laboratory Technics: *Platelet counts* were done by the direct phase microscope technic of Brecher and Cronkite.[5] *Factor V levels* were assayed by the method of Borchgrevink, Pool and Stormorken.[4] This assay had to be modified in the case of heparin blood, since small amounts of heparin affect the assay. On the other hand, the assay is not altered by relatively large amounts of the heparin neutralizer Polybrene®.* Heparin was thus neutralized in

* Abbott Laboratories, North Chicago, Illinois.

the plasma before assay by adding 100 micrograms of Polybrene® per ml. plasma, more than enough to neutralize any heparin present. To prevent coagulation with resulting loss of Factor V, the Polybrene® was diluted in the citrated saline solution required for the Borchgrevink method. A Polybrene® concentration of 100 micrograms per ml. of solution was used so that equal volumes could be mixed with the plasma to be tested. Repeated simultaneous assays of blood collected in citrate and in heparin proved that this technic gave the same answer as the unmodified assay. *Plasma hemoglobins* were measured by the

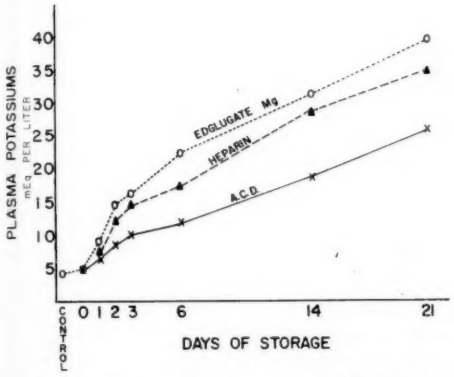

FIG. 8. A comparison of changes in plasma potassium levels on storage of blood in ACD, heparin and Edglugate-Mg.

benzidine technic of Crosby and Furth,[8] *plasma potassium levels* with a Coleman flame photometer, and *blood pH* at 37 C. with a Beckman model G *p*H meter using a blood electrode which prevented exposure to air. *Protamine titrations* were done by a method previously described.[23]

All values reported for the *in vitro* studies (except *p*H) have been corrected for dilution by the original anticoagulant as well as any further dilutions before assay to permit direct comparison among the different series of stored blood.

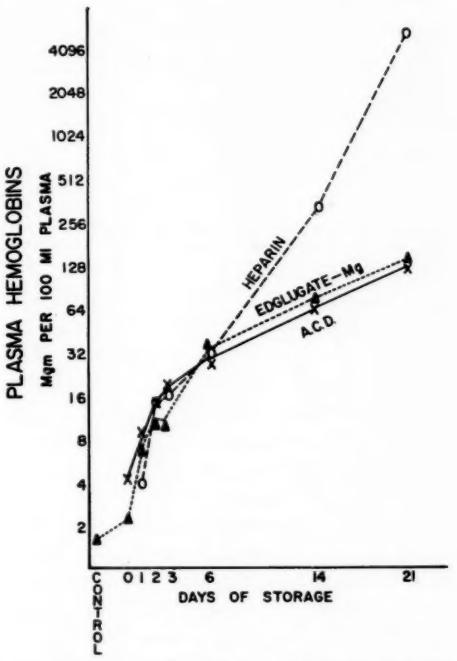

FIG. 9. A comparison of changes in plasma hemoglobin levels on storage of blood in ACD, heparin and Edglugate-Mg. (Note change to log scale on vertical axis of this figure.)

Results

Figures one through five show the changes that occurred in ACD blood during a 21-day period of storage. The vertical lines represent one standard deviation above and below the mean. It is apparent that there

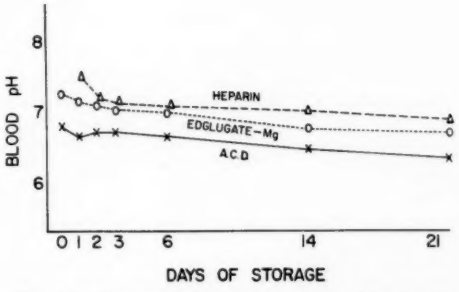

FIG. 10. A comparison of changes in blood *p*H on storage of blood in ACD, heparin and Edglugate-Mg.

TABLE 1. In Vitro *Changes in Heparin Blood on Storage*

	Day 1	2	3	6	14	21
Platelets x 10^3		225±75*	216±85	132±60	75±51	55±3
Factor V %		106±28	94±30	78±32	49±19	26±9
Potassium mEq/L	6.9±1.6	12.2±3.2	14.3±3.0	17.0±2.8	28.3±3.1	34.6±4.0
Plasma Hemoglobin mgm %	4±3	15±10	17±10	31±14	333±173	1536±2.8
*p*H	7.50±.14	7.18±.03	7.12±.03	7.06±.05	6.98±.08	6.87±.07

* Standard Deviation

is no significant difference in the results whether the blood had been collected with the aid of vacuum or by gravity. The average plasma hemoglobin levels in the vacuum bottles were higher at first (although the difference is not statistically significant). Within 48 to 72 hours the difference had disappeared. Within each series there was a wide range of difference between individuals in *Factor V levels, platelet counts,* and *plasma hemoglobins.* This accounts for most of the wide standard deviations. Some of the variation in platelet counts was undoubtedly due to difficulty in obtaining adequate mixing of the large volume of blood. These variations are apparent in the other series of bloods to be reported below, and point out the inadequacy of comparisons between small numbers of bloods.

Figures six through ten show the changes in blood collected in heparin and Edglugate-Mg, as compared with the ACD gravity series. Only the mean values are plotted to avoid confusion; the standard deviations may be found in Tables 1 and 2. It is apparent that *platelets* disappeared from heparinized blood much faster than they did in ACD. *Factor V* levels were the same in both solutions. *Blood pH* was higher in the heparinized blood throughout, which is to be expected in view of the fact that ACD is deliberately designed to lower the blood *p*H. The rate of fall of *p*H was initially faster in the heparinized blood, but after 48 hours the curves are nearly parallel. *Plasma potassiums* and *hemoglobin* levels were initially in the same range in ACD and heparinized blood, but became significantly higher in the heparinized blood on storage. Potassium levels were obviously rising faster within two days, plasma hemoglobins after six days.

It has occasionally been stated that heparin disappears from blood during prolonged storage. This opinion appears to be based on the fact that with borderline concentrations of heparin clots may eventually appear to a varying degree. Our heparinized bloods remained liquid during the 21-day period of storage, although increasing numbers of very small clots were

TABLE 2. In Vitro *Changes in Edglugate-Mg Blood on Storage*

	Control	Day 0	1	2	3	6	14	21
Platelets x 10^3	272±69*	227±65	164±60	149±25	138±30	120±29	96±42	91±22
Factor V %	100±23	42±13	32±7	28±5	31±6	20±8	5±5	3±5
Potassium mEq/L	1.2±0.1	1.8±0.1	8.9±0.9	11.1±3.1	16.0±2.5	22.2±5.8	31.2±6.4	39.3±6.1
Plasma Hemoglobin mgm %	2±2	2±1	7±3	12±5	11±6	34±22	78±48	143±88
*p*H		7.23±.05	7.15±.02	7.11±.02	7.05±.04	6.99±.04	6.77±.04	6.69±.04

* Standard Deviation

encountered during the last two weeks. In other experiments heparin remained unchanged, as measured by protamine titration, in two bloods stored for 21 days, one stored for two months, and one stored for six months. During the first 21 days of storage repeated protamine titrations showed progressively shorter clotting times in the tubes in which heparin had been neutralized by protamine. It thus appears that heparin is very stable in blood on prolonged storage, but that its effect may be overcome to a varying degree by progressive change in blood coagulation factors. The most likely explanation is evolution of thromboplastic materials from the breakdown of platelets and red blood cells, but changes in other pro- and anticoagulant factors may play a role.

In the Edglugate-Mg series the fall in the *platelet count* also occurred at a faster rate than it did in ACD. *Factor V* activity in Edglugate blood was low when first assayed, one hour after collection. On further storage over a 21-day period, however, the remaining activity decreased at a rate no faster than occurred in ACD or heparin blood. *Potassium* levels rose faster and higher in the plasma of Edglugate-Mg blood than they did in heparin. *Plasma hemoglobin* levels, however, ran parallel to those in ACD, and did not demonstrate the high rise that occurred in heparin. The *pH* changes paralleled those in ACD, but were higher for reasons already discussed.

In vivo studies were limited to a comparison of the effects of heparin blood which was four hours old with that after 24 hours of storage. Table 3 shows that there was no significant difference in the platelet counts in the donor heparin blood mixed in the pump-oxygenator, whether it was four or 24 hours after collection. If there was any difference in platelet viability, it is not reflected in the course of platelet counts in the patients (Table 4). (A few experiments, as yet unpublished, indicate that dog donor platelets do survive heart-lung bypass to some extent.) Table 5 shows the heparin levels remaining in patients after bypass as measured by protamine titration. There is no evidence that heparin was disappearing in the donor blood during the first 24 hours after collection.

TABLE 3. *Platelet Counts in Mixed Donor Blood in Pump Oxygenator Before Bypass (all counts x 10^5)*

	Four hour old blood	Twenty-four hour old blood
	140	184
	170	165
	220	208
	189	132
		214
		84
		185
		152
		136
		197
		193
Averages	180	169
Standard Deviation	±34	±39

TABLE 4. *Effect of Age of Donor Blood on Platelet Counts During Open Heart Surgery*

Age of donor blood Number of cases	Four hours 29		Twenty-four hours 10	
	Platelet count x 10^5	Standard deviation	Platelet count x 10^5	Standard deviation
Before surgery	266	±133	284	±60
Before bypass	233	±83	220	±50
After bypass	143	±38	130	±40
1 hour later	171	±44	190	±51
2 hours later	211	±38	246	±61

TABLE 5. *Protamine Titrations after Bypass*

Age of blood	No. of cases	Protamine requirements*	S.D.
4 hours	18	27	±7
24 hours	13	25	±7

* Minimum number of micrograms per ml. of blood required to reduce clotting time to normal range.

Discussion

The results presented above represent only the beginning of an attack on the vastly complex problem of what is necessary to preserve the characteristics of fresh blood longer, with particular reference to the provision of blood for open heart surgery. The standard ACD solution has been used as a basis for comparison. The results reported here on the changes that occur in ACD blood on storage are in general agreement with previous reports in the literature. Changes in plasma potassium on storage have been remarkably consistent in a wide variety of citrate anticoagulants and in different containers,[2, 9, 25, 26, 30] the levels being slightly lower in the presence of added glucose. Only a few reports of changes in plasma hemoglobin levels in ACD blood have been made in recent years since this test shows poor correlation with red cell viability.[25] It has been used here because of its importance in heparinized blood and in the changes induced by extracorporeal bypass. The results reported above fall in the mid-range of those published. The trend of *p*H levels here is consistent with other studies;[11, 25, 30] but values are somewhat lower, since escape of carbon dioxide was not permitted. The rate of disappearance of Factor V in ACD blood here agrees with the findings of Rapaport[24] and of Mustard;[18] but Kettenborg's series[12] showed a more rapid fall. Platelet counts in this study persisted at higher levels than in earlier reports in which citrate without glucose was used as the anticoagulant; however, they also remained better than in Muether's series[16] in which ACD blood was collected in plastic bags. No explanation for this is apparent. The results here do agree with those of Mustard[19] when he collected blood in ACD in siliconed bottles.

It is not clear at this point how important it is that the blood be collected in containers with non-wettable surfaces and without the assistance of vacuum in order to preserve platelet viability and the level of labile clotting factors. Krevans[14] has reported that it makes little difference in the viability of *dog* platelets collected in ACD whether the container is a plastic bag filled by gravity or an uncoated glass bottle filled by vacuum. The only previously reported attempt to compare the effect on human platelets of gravity and vacuum collection was that of Mustard.[19] His studies, like those here, were confined to *in vitro* tests. He found better persistence of platelets in the vacuum series than in the gravity one, which is an unexpected result; but most of his gravity bottles were filled through red rubber donor blood tubing, and this may be a partial explanation. The findings in the present study indicate that there is no difference in the changes that occur in ACD blood in siliconed bottles whether or not vacuum was used to assist the collection. Any conclusion to be drawn from this would be necessarily very tentative, since under the best conditions the proportion of platelets which will survive in the recipient after transfusion falls off rapidly to zero in the first few days of storage.[3] Nonetheless, Mustard's demonstration[17] that there is a correlation between the initial levels and survival of platelets *in vitro* and their ability to persist in the circulation of the transfused patient suggests that the *in vitro* data here may be an indication of relative changes in viability.

Recent success in the transfusion of viable platelets and labile coagulation factors is considered to be due in part to

avoiding initiation of coagulation by careful venepuncture and use of non-wettable surfaces. Rapid gentle mixing with the anticoagulant is of obvious importance. The avoidance of undue trauma to the blood and of foaming, especially by vacuum, is particularly stressed. There is no doubt, of course, that beginning coagulation will accelerate the loss of platelets and labile coagulation factors; and it is also true that coagulation is speeded up by uncoated glass, tissue juices (thromboplastin) and foaming of blood. However, when a large volume of blood is rapidly mixed with anticoagulant, the coagulation process is interrupted before much of the blood has ever been in contact with the surface of the container. It has previously been shown[21] that when heparin is used as the anticoagulant, the fall in the platelet count is increased in proportion to the amount of contact the blood has had with the container surface before heparin has had time to inactivate the process completely.

It would seem to be of obvious importance that blood be collected with expendable plastic donor tubing, since blood remains in contact with this surface for 5 to 10 minutes during the collection period. As previously demonstrated, blood in plastic donor tubing removed from the donor's arm at the end of collection will not clot for a period of approximately 90 minutes.[22]

Krevans' results and those reported above suggest that collection of blood with the aid of vacuum may not speed up the loss of platelet viability and labile coagulation factors. No evidence has ever been presented to the contrary; the recommendations to avoid vacuum usually refer to papers of Stefanini,[28, 29] who provides no data on this point.

Since heparin must be used to anticoagulate the blood required during open heart surgery, current practice is to collect the required blood with heparin as the sole anticoagulant. While data have been scarce, it has been apparent that such blood deteriorates rapidly. Most teams use this blood within 24 hours of collection, a few have used 48-hour-old blood, and some still insist on blood collected the same day. The results reported here show clearly that blood collected in heparin loses platelets quicker and has faster rises in plasma potassium and hemoglobin than ACD blood. The deviations are marked after 72 hours, and suggest that the present limits of storage of heparinized blood cannot be much extended. On the other hand there is no available information as to the relative viability of platelets in reasonably fresh heparinized blood compared with ACD. The *in vivo* studies reported here suggest that if there is any difference in viability of platelets in 24-hour-old as compared with four-hour-old heparin blood, it has no significant effect on the platelet count of the patient. It would seem apparent that 24-hour-old blood is as safe to use as blood collected the day of operation, and studies of heparinized blood stored for slightly longer periods appear worth pursuing.

It should be pointed out that the heparinized blood used in the studies reported here had no added glucose, which is known to be necessary for the prolongation of red cell viability in citrate solutions. No information is available to indicate whether glucose would minimize the changes seen here. Abbott[1] has also reported studies on the storage stability of heparinized blood. He compared a wide variety of solutions, and did not demonstrate any protective effect due to glucose. The value of his data, however, is limited by the small number of bloods in each of the series he compared.

Edglugate-Mg was introduced as an anticoagulant by Smith and Brown[27] in the hopes of overcoming some of the disadvantages of using heparinized blood. The difficulty with heparinized blood is its limited

period of storage, which necessitates arranging for the bleeding of type-specific donors shortly before operation. A last minute cancellation of surgery may result in the need to discard all the heparinized blood collected. It is not safe to release it for routine transfusions, even if still fresh, since the heparin in it would make it unsafe for patients who were bleeding or had a tendency to a hemorrhagic state. The Duke group considered the possibility of collecting and storing blood in the routine ACD solution, and then just before surgery adding heparin and neutralizing the citrate with calcium. There were a number of theoretical objections to this idea. ACD solution (NIH Formula B) adds 120 ml. of water and a considerable amount of sodium to each unit of blood. It might be expected that this would be a dangerous load for patients who may be in cardiac failure. The acid content is high. The amount of calcium required to neutralize the citrate is unpredictable since citrate is metabolized by the patient at varying rates. It was therefore considered worth exploring the use of EDTA, a calcium binder more potent than citrate. EDTA could be used in a smaller volume of solution for this reason, containing less sodium. Moreover, EDTA is not metabolized by the body, and the amount of calcium required to neutralize it in the *in vitro* container is all one needs to know. The problem of neutralizing the calcium exactly in these patients is of very great importance, since they have a considerable tendency to cardiac arrythmias and standstill following surgery, and improper blood levels of ionized calcium would aggravate this.

Edglugate-Mg, however, introduces new problems. Most important is the fact that potassium leaks from the red cells into the plasma during storage at a rate far faster than occurs in ACD. Infusions of large volumes of blood with high potassium levels in the plasma can by itself result in cardiac arrythmias and standstill.[15] Preliminary reports on the use of Edglugate Mg blood indicate that the patient can tolerate the levels of potassium achieved within five days of storage, but suggest that it would be unsafe to go beyond that limit. The data reported here, which agree with previous results,[27] indicate that potassium rises faster in the plasma of Edglugate Mg blood than in heparin.

The labile coagulation factor, Factor V, has been known for some time to lose activity rapidly in EDTA plasma. Following the work of Zucker,[31] the Duke group incorporated magnesium into their formula to minimize this. Despite this, the data here show that Factor V activity is very low within an hour after collection of the blood in Edglugate-Mg. Unexpectedly this rapid rate of loss did not persist, and further changes during the 21-day period of storage paralleled those in heparin and ACD. Smith[27] has shown that much of the Factor V loss is only apparent, and can be restored by dialysis against plasma from outdated bank blood. His results have been confirmed in this laboratory. It is probable then that changes in coagulation factors need not be considered when deciding on the desirability of Edglugate-Mg blood.

It has been suggested that EDTA is a better anticoagulant for platelet preservation than ACD since its greater inhibition of platelet clumping permits the preparation of platelet concentrates with a larger proportion of viable platelets.[3] The superiority of EDTA may not apply to situations where the platelets are transfused in whole blood rather than as concentrates; in any event our data indicate that *in vitro* survival of platelets on storage is definitely better in ACD.

Plasma hemoglobin levels in Edglugate-Mg followed the course of the ACD blood. It is of considerable interest that potassium leaked from the red cells of Edglugate blood faster than from heparinized blood, whereas the reverse was true for hemoglobin. This dichotomy in the rate of loss

of these two substances has been reported before. It may be significant that the two solutions with the lowest plasma hemoglobin levels were the two which contained glucose. The effect of glucose on the storage stability of heparinized blood needs to be explored further.

The final objection to the use of Edglugate-Mg is the fact that EDTA in sufficient dosage is toxic to the kidneys. The amount which would be left in the patient after open heart surgery was originally believed to be below the danger point. However, recent attention has been drawn to the current use of pump-oxygenators in some areas whose priming volume is so large that toxic doses of EDTA could conceivably be left in a small patient. For this reason our supplier has stopped the manufacture of Edglugate-Mg even on an experimental basis.

Before leaving the subject of Edglugate-Mg, however, it should be pointed out that it is quite probable that the detrimental effects of EDTA on the kidney, on Factor V levels, and on the rate of potassium leakage from the red cell is based on the chelation of important cations. If these cations could be identified, added to the formula, and not displace calcium in the process (and if it is not calcium ion itself[6]), then a new formula based on EDTA might be worth exploring.

The most recent development in the field of providing blood for open-heart surgery has been the report of Foote, Trede and Maloney[10] that the theoretical objections to the use of heparinized recalcified ACD blood mentioned above did not appear to play a significant role in practice. The Los Angeles group has been using bank blood stored in ACD up to five days in this manner in a large number of human open heart operations without harmful effect.

Addendum

Since this manuscript was submitted for publication another report has been published by Lobpreis, *et al* * which confirms the above conclusions that 24-hour-old heparinized blood is safe for use in open heart surgery. There are two discrepancies between the work of Lobpreis and the results reported above. They report a much faster loss of platelets in stored heparinized blood, using an indirect method of counting. They also report a disappearance of approximately one quarter of the heparin activity as measured by protamine titration in the first 36 hours. The discrepancy here probably lies in the choice of end point. Lobpreis took as the end point the smallest amount of protamine required to restore the clotting time to 15 minutes or below. This end point would be expected to shift to a smaller value if the clotting time of completely neutralized blood shortened.

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Electrophoretic Analyses of Red Cell Hemolysates Before and During Storage of Blood from Diseased Individuals*

CLAUDE A. SMITH, M.D., ALFRED CHANUTIN, PH.D.

From the Department of Biochemistry, School of Medicine, University of Virginia, Charlottesville, Virginia

The bloods of six healthy young male students were stored anaerobically in the cold and at intervals the stroma-free red cell hemolysates were analyzed in a cacodylate buffer at *p*H 6.5 and .05M concentration. From these data, a control range was established.

Results are presented which show the effect of anaerobic storage on the B/2 values of red cell hemolysates of the bloods of 23 patients with a variety of diseases. In 21 cases, the B/2 values decreased rapidly or showed an initial increase followed by a decrease. Attempts to relate the type of storage curve to specific diseases were unsuccessful. It is concluded that water-soluble protein components of the red cells may be altered by disease.

IT HAS been shown[1, 2] that stroma-free human red cell hemolysates in cacodylate buffer yielded an electrophoretic pattern containing three components which were designated as f, A and B. It was found that the concentration of Component B decreased at a relatively rapid rate after the first week and subsequently at a slower rate when the bloods of healthy individuals were stored under anaerobic conditions.[2] Preliminary evidence[1] showed that the B/2 storage curves of the bloods of diseased individuals varied considerably from the control curves. In these earlier experiments, the blood was stored in cotton-plugged flasks (aerobic), only a few analyses were made on each blood and the patterns were obtained with the Philpot-Svensson cylindrical lens method.

This present study was undertaken to evaluate the effect of anaerobic storage of bloods of patients on the concentration of Component B of the red cell hemolysates.

Methods

Preprandial blood was collected under sterile conditions in an evacuated rubber-stoppered serum bottle which was immersed in ice water and stirred continually during phlebotomy. Acid-citrate-dextrose (ACD, NIH, Sol. B) was employed as collecting medium. Four ml. aliquots were transferred to glass-stoppered tubes which were stored in a cold room at 4 C. Centrifugation, washing, lysing, clarification and dialysis against cacodylate buffer were conducted at 4 C.

After centrifugation of an aliquot, the plasma and buffy coat were removed and the red cells were washed three times with 0.85 per cent NaCl. Lysis was accomplished by adding five volumes of water to one volume of red cells and shaking vigorously at intervals during a 30-minute period. The hemolysate was clarified by centrifugation for 30 minutes at 10,000 rpm. in a Servall SS-1 centrifuge. The hemoglobin was determined[3] and 5 ml. of a 1 per cent hemoglobin-containing solution was prepared for analysis. This solution was dialyzed against 0.05M sodium cacodylate-cacodylic acid buffer (*p*H 6.5) which was changed three times during an 18 to 24-hour period.

Electrophoresis proceeded for 90 minutes with closed electrodes in a Klett-Tiselius apparatus using a 2 ml. micro-cell. A photograph was taken by Longsworth's scanning technic[5] immediately after the current was shut off. A second photograph was obtained after the refractive bar of the A

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FIG. 1. Electrophoretic patterns of red cell hemolysates of a blood from a healthy young man. Aliquots of the blood were stored at 4 C. in glass-stoppered tubes. The black area represents B/2. Due to the sharpness of Component A, only a portion is shown.

boundary had cleared. The area of the B/2 was obtained from the ascending pattern of the first plate and the total area of the pattern from the second plate. The patterns were magnified four times and traced on paper. The area designated as B/2 was delineated by dropping a line from the peak of the B boundary to the base line and the area measured by planimetry.

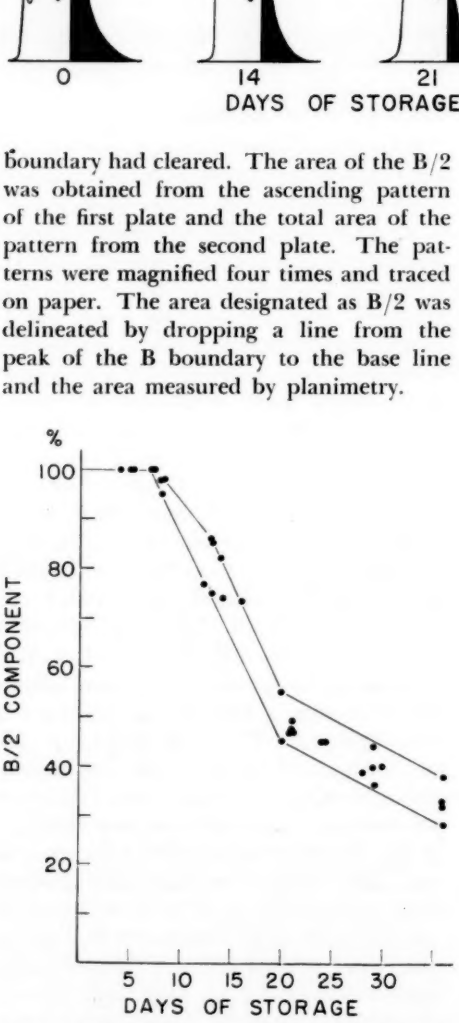

FIG. 2. Effect of storage on the B/2 concentrations of the bloods of six healthy young men. The results are expressed as percentage of the initial B/2 value. These data serve to define the control range which is shown as a shaded area in succeeding figures.

Experimental Results

Controls: In a previous paper,[2] it was shown that the Component B concentration remained elevated for a comparatively long time when blood was stored under aerobic conditions but decreased much sooner if storage was conducted under mineral oil, in glass-stoppered tubes or in standard glass collection bottles. The electrophoretic patterns of stroma-free hemolysates, prepared from the blood of a healthy young man, are shown after 14, 21 and 28 days' storage at 4 C. in glass-stoppered bottles (Fig. 1). A satisfactory analysis of the two main Components A and B was not possible due to the asymmetry of the boundaries. The B/2 area, which is expressed in percentage of the total area of the pattern, appeared to be the most reliable measure of this material under these conditions. A scatter diagram (Fig. 2) shows the changes in the B/2 values during the storage of bloods of six healthy male medical students. These results are plotted in terms of the percentage of the initial B/2 values which varied between 16.2 and 21.3 per cent of the total area. It was found that samples of blood drawn at varying intervals from the same individual yielded reproducible B/2 storage curves. The curves for the bloods of healthy males, ranging in age from 30 to 60 years, fell within the control range obtained for men about 20 years of age. Bloods of healthy females and children were not studied.

Disease States: The B/2 storage curves for 23 patients are arbitrarily divided into three groups: (1) no increase in B/2 values (Fig. 3); (2) increases between 100 and 130 per cent above initial values (Fig. 4); and (3) increases above 130 per cent (Fig. 5).

In group 1, four of the six curves are definitely outside the control range and are characterized by immediate decreases in B/2 values. The initial B/2 values vary between 17 and 24 per cent of the total pattern areas.

In group 2, the maximum increases are reached after about one week's storage for six cases and after about two weeks for three cases. In all but one instance, the B/2 values decrease rapidly after the peak concentration is reached. The exception is seen for the stored blood of a case of pulmonary fibrosis (curve 1) with an initial B/2 value of 31.5. In the remaining cases the initial B/2 values vary between 15.3 and 21.6.

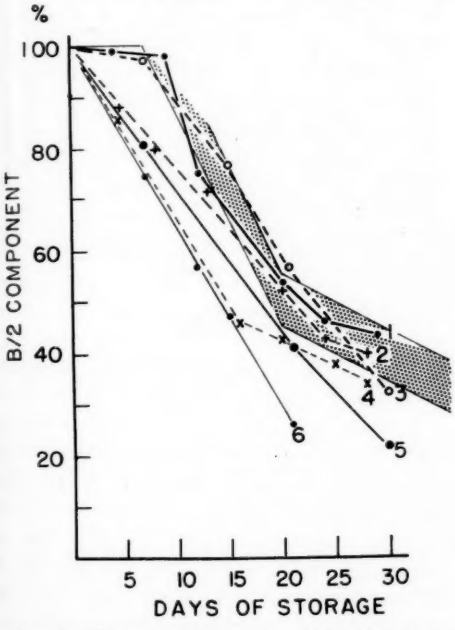

FIG. 3. The storage curves in which there is no increase above the respective initial B/2 values (Group 1). The numbers in parenthesis represent the 0 day concentration of Component B/2: 1—(18.5) diabetic acidosis; 2—(18.8) metastatic mucin-producing adenocarcinoma of bowel; 3—(24.0) pulmonary fibrosis; 4—(21.0) papillary adenocarcinoma of left thyroid; 5—(17.0) sarcoidosis; 6—(21.5) spherocytic anemia.

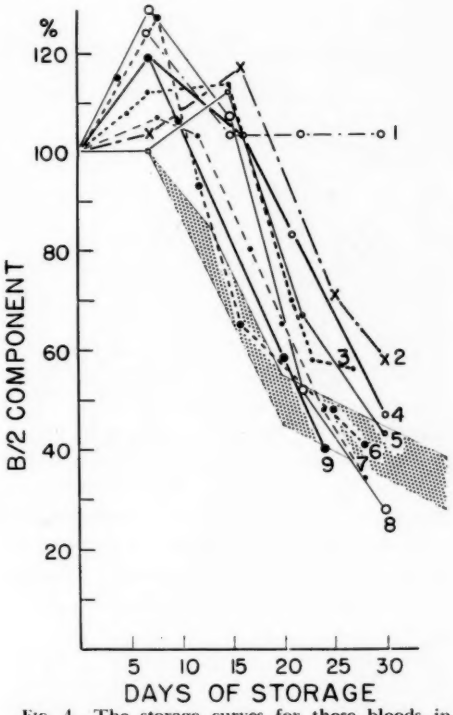

FIG. 4. The storage curves for those bloods in which there are increases in the B/2 concentrations varying between 100 and 130 per cent of the 0 day value (Group 2): 1—(31.5) pulmonary fibrosis; 2—(15.7) multiple myeloma; 3—(20.8) anemia of unknown etiology; 4—(15.8) Laennec's cirrhosis; 5—(21.6) arteriolar nephrosclerosis; 6—(17.7) malnutrition; 7—(16.2) exogenous obesity; 8—(17.3) secondary amyloidosis; 9—(15.3) Laennec's cirrhosis.

In five of the eight curves in group 3, it is observed that the maximum increases are between 164 and 187 per cent above the initial B/2 concentration. In all cases, the peak values are attained between the seventh and sixteenth days of storage. Inspection of the curves reveals that the B/2 values for patients 1, 2 and 3 are above their respective initial values after one month's storage and at this time the B/2 values for the remaining five bloods are

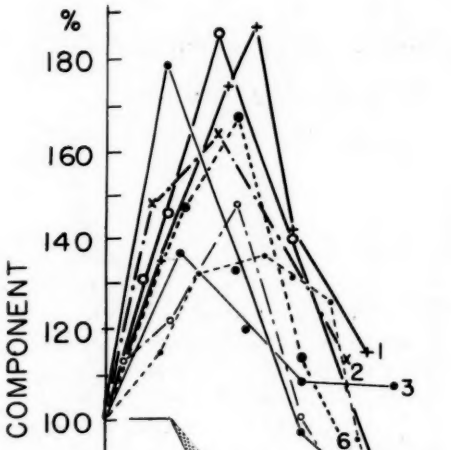

FIG. 5. The storage curves in which there are increases in the B/2 concentrations greater than 130 per cent above the respective initial concentrations (Group 3): 1—(10.4) Laennec's cirrhosis; 2—(11.1) severe metabolic alkalosis-vomiting; 3—(8.5) acute leukemia, type unspecified; 4—(11.1) iron deficiency anemia; 5—(12.2) multiple myeloma; 6—(8.7) chronic lung disease; 7—(9.8) diabetes mellitus; 8—(15.8) multiple myeloma.

well above the control levels. In all but one of these cases, the initial B/2 values are low with a range of values varying between 8.5 and 12.2.

Discussion

The significance of the changes observed in the concentrations and the shapes and mobilities of the components of the red cell hemolysates during storage of blood cannot be understood until the materials are separated and characterized. Component A is undoubtedly hemoglobin as shown by a comparison of electrophoretic patterns of crystalline hemoglobin and red cell hemolysates. Component B has not been separated but indirect evidence indicates that it is a protein-phosphate complex. After incubation of a hemolysate with alkaline phosphatase for one hour, only a trace of Component B is present. The nature of Component f is not known. This problem is complicated further by the fact that a number of heme and non-heme proteins have been reported to be present in the red cell hemolysate.[4]

It was hoped that the B/2 storage curves for a given disease would be similar but inspection of the curves obtained with three cases each of chronic lung disease, Laennec's cirrhosis and multiple myeloma, failed to yield uniform changes. An attempt to correlate the degree of anemia and the type of storage curve was unsuccessful. Because of the complex nature of the storage curves, no conclusions can be drawn concerning the significance of the results.

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Specificity of the Agglutinating Phase of Immunohematologic Reactions with Non-Saline Agglutinating Antisera*

MORTON D. PRAGER, PH.D.

From the Wadley Research Institute and Blood Bank, Dallas, Texas

When mixtures of stained and unstained human red cells of different antigenic composition are mixed with homologous non-saline agglutinating antisera in a small Petri dish, two distinctly different clumps of cells are formed. This observation suggests that the agglutinating phase of the reaction is specific in accord with the Marrack hypothesis and further implies that these antibodies are not univalent but are bi- or polyvalent. As a result of formation of a single cell clump, application of this technic has led to the discovery of a serum containing a heretofore unrecognized anti-CD antibody.

IT IS convenient to consider that immunohematologic reactions, like other immunochemical reactions, occur in two phases: (1) primary interaction of antibody with homologous red blood cells, and (2) the coalescence of these primary reaction products into visible agglutinates. While the specificity of the first phase may be considered a fundamental theorem of immunochemistry, there has been disagreement over the specificity of the second phase. Topley, Wilson, and Duncan[9] were the first to investigate the question with mixtures of cell types (bacterial) and homologous, non-cross reacting antisera. By finding that two distinct clumps of cells formed, each containing a single cell type, they supported the concept of specific agglutinating phase. Their evidence was thus in accord with the Marrack lattice theory and opposed to the idea of non-specific second phase suggested by Bordet. Other workers who applied this technic to a variety of mixed cell populations have not always come to the same conclusion. Hooker and Boyd[5] stated their belief that the factor controlling the number of clumps was the relative rates of agglutination of the two kinds of cells; *e.g.*, if one cell type agglutinates considerably faster than the other, then two clumps will always form. Pauling and his collaborators,[7] on the other hand, claimed that the appearance of two distinct clumps could hardly be explained by the action of nonspecific forces, and that when one clump was found, it might result from mechanical entrapment of smaller specific clumps. Most workers, including those who earlier held the opposing view (Boyd[2]), now agree that the bulk of the available evidence supports the Marrack theory and the concept of specific second phase.

This study is concerned with an extension of the use of agglutination of mixed cell populations to non-saline agglutinating antisera and the implications of the findings relative to the valence of these antibodies. A preliminary report of this work has been given.[8]

Materials and Methods

Erythrocyte donors used in this study were from the donor lists of the Wadley Research Institute. Antisera were for the most part from donors in this Institute: specificities included anti-A, anti-B, anti-D, anti-E, anti-c, and anti-CD; anti-C was obtained from the Milwaukee Blood Center. The Rh antisera (Wadley) have been examined on numerous occasions in these

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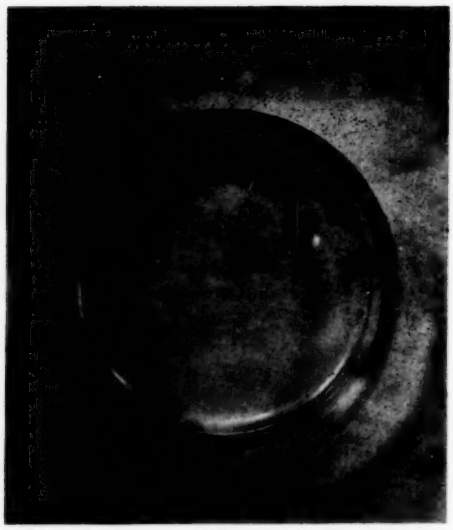

FIG. 1. Photograph of the two distinct agglutinates typically formed from a mixture of non-saline agglutinating Rh antibodies of different specificities and their homologous papain-treated erythrocytes. One group of red cells is stained with methylene blue so that the two clumps may be differentiated.

laboratories and have never been observed to possess saline agglutinating activity.

A number of dyes were examined for their ability to stain red cells sufficiently to give color differentiation. These include brilliant cresyl blue, wool violet, titan yellow, sudan black, neutral red, Janus green, fast green, light green, methyl green pyronine, carbol gentian violet, potassium ferricyanide plus potassium cyanide (to prepare cyanmethemoglobin), Wright's stain, May-Greenwald's stain, and Loeffler's methylene blue. Only the latter proved useful. To obtain adequately stained cells, 4-5 drops of Loeffler's methylene blue (30 ml. 1.5 per cent methylene blue in 95 per cent ethanol, and 100 ml. 0.01 per cent aq. KOH) was added to 5 ml. of a heavy saline suspension of cells. After standing at 25 C. for 2 hours, the cells were washed with saline until no further dye was removed.

The details of the mixed agglutination procedure were worked out with anti-A, anti-B, group A_1 and group B erythrocytes, one or the other of which was stained. Two drops of each cell suspension (2 per cent) was placed in a small Petri dish with two drops of each antiserum. The dish was incubated at room temperature with gentle, intermittent agitation. When using non-saline agglutinating antisera (*i.e.*, sera with "cryptagglutinoid" or "incomplete" antibodies only), red blood cells were first stained and then treated with papain by the modification of Hill, Kurtar and Soule[4] before being placed in the Petri dish mixture. With the Rh antisera, incubation was at 37 C. In one group of experiments the agglutination rate was adjusted so as to be the same for each antiserum with its homologous cells. This was accomplished by diluting the serum so that incipient agglutination of a 2 per cent red cell suspension on a microscopic slide was 50 ± 2 sec.

Results

In the experiments with group A_1 cells, methylene blue stained group B cells (or *vice versa* with respect to staining), anti-A, and anti-B, two distinct cell clumps formed, one red and one blue. Intermittent agitation is of importance, particularly in the late phases of agglutination, in preventing entrapment of cells of one type in a clump of cells of the other type.

When this technic was applied to reaction of non-saline agglutinating antisera of the Rh system with papain-treated cells, two clumps were again observed (Fig. 1). The formation of small clumps of cells was observed in the first couple of minutes, but 20-30 minutes were required for the cells to come together into two clumps. Typical findings with a variety of cell types and antibodies of several specificities are summarized in Table 1. A number of entries represent multiple experiments with cells of the more common genotypes having been obtained from several different donors. The findings may thus be considered general and not limited to partic-

TABLE 1. *Agglutination of Mixed Populations of Papain-Treated Erythrocytes with Non-Saline Agglutinating Antisera*

	RBC probable genotype		Antibody specificity	Number of clumps
	Stained	Unstained		
1	*CDe/CDe*	*cde/cde*	D and c	2
2	*cde/cde*	*CDe/CDe*	D and c	2
3	*CDe/CDe*	*CDe/CDe*	D	1
4	*cde/cde*	*cde/cde*	c	1
5	*cDe/cde*	*Cde/cde*	C and D	2
6	*cdE/cde*	*CDe/CDe*	D and E	2
7	*CDe/CDe*	*cdE/cde*	D and E	2
8	*cdE/cde*	*cdE/cde*	E	1
9	*cDe/cde*	*Cde/cde*	C, D, and CD	1

ular donors. Entries 1 and 2 and 6 and 7 show that two clumps form irrespective of which cell type is stained in the mixture. Entries 3, 4, and 8 give evidence that the dye itself does not interfere in such a way as to prevent stained and unstained cells from agglutinating together. In several experiments, antisera were diluted so that agglutination of the two types of cells would proceed at the same rate. When mixtures like those of entries 1 and 6 (Table 1), for example, were prepared with these adjusted antisera, two clumps were still observed in each instance.

A particularly interesting serum is that described in entry 9, Table 1. Evidence for the presence of anti-CD in this serum in addition to anti-C and anti-D rests on typical adsorption-elution experiments. The serum was first adsorbed with C+ D− red cells; an eluate was then prepared which was next adsorbed with C− D+ cells. An eluate from the latter reacted positively with all C+ and D+ red cells but with no *cde/cde* erythrocytes.* This serum repeatedly gave only one cell clump. In a Petri dish experiment set up simultaneously with the same erythrocytes but with anti-C and anti-D from two different serum donors, two cell clumps formed (one red and one blue).

A number of attempts were made to apply this technic to agglutination in colloidal medium to determine if the buildup of agglutinates in such medium was also specific. These experiments were performed with several concentrations of albumin, polyvinylpyrrolidone, or mixtures of albumin and AB serum. However, the medium was either too viscous for good agglutinates to form (without centrifugation) or the cell clumps were too loose and small to permit color differentiation.

Discussion

By finding two clumps of cells in the mixtures of A_1 and B red cells with anti-A and anti-B, one may conclude that the agglutinating phase of the reaction is specific in accord with the Marrack hypothesis. This conclusion agrees with that of McKerns and Denstedt[6] who also used human erythrocytes to study this point. They made their color differentiation by converting the hemoglobin in one group of cells to sulfhemoglobin. Cotterman[3] in studies with stained erythrocyte ghosts also observed two clumps and reached the same conclusion.

* I am indebted to Dr. David Soules for the adsorption studies which showed the presence of the three antibodies in this serum.

The unique feature of this study is the application of this technic of observing agglutination of mixed cell populations to non-saline agglutinating antisera. Here again, with the formation of two clumps of cells, one may reasonably conclude that the agglutinating phase of the reaction has specificity. In this regard, the experiments involving the use of antisera for which the rates of agglutination were identical are important in overriding the earlier, valid objections of Hooker and Boyd[5] that two clumps might form not necessarily as a result of specificity but simply as a result of the two reactions proceeding at different rates. The suggestion has frequently been made that whereas saline agglutinins are bi- or polyvalent, perhaps the non-saline agglutinating antibodies are univalent. In view of the demonstrated specificity of the agglutinating phase of the reaction of papain-treated red cells with non-saline agglutinating antisera, this hypothesis no longer seems reasonable.

The serum of entry nine (Table 1) turned out to be most interesting. When only one cell clump formed in repeated experiments, it was suspected that there might be present antibodies in which C and D specificities were linked in the same molecule. The adsorption experiments are in accord with this hypothesis. As there were no undoubted G+ cells available, it cannot be stated whether the two specificities are independent of each other but are simply linked by virtue of being in the same molecule or whether they together contribute to the formation of a new, additional specificity; namely, the G, first described by Allen.[1] This demonstration is probably the first in which this kind of dual specificity antibody has been uncovered from observation of its effect on a mixed cell population.

Acknowledgment

I gratefully acknowledge discussions with Dr. C. W. Cotterman which stimulated this study, the interest of Dr. J. M. Hill and his aid in the acquisition of antisera, and the valuable technical assistance of Miss Ina Claire Atkins.

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An Evaluation of the Plasmacrit Test for Syphilis in a Clinical Laboratory*

ALBERT BALOWS, PH.D., JAMES T. MCCLELLAN, M.D., SUE ALLEN, B.S.
Section of Clinical Pathology, The Lexington Clinic, Lexington, Kentucky

The plasmacrit (PCT) is a modification of the VDRL slide test for syphilis in which plasma instead of serum is used as the source of reagin. The plasma inhibitory component is neutralized by using 10 per cent choline chloride as the diluent in the preparation of the VDRL antigen suspension. We performed the PCT and VDRL simultaneously on the plasma and serum of 2,458 individuals. There was complete agreement between the two tests in 99.4 per cent of the total number tested. Different results were obtained with 15 specimens and with only one exception, these results indicated a greater sensitivity for the PCT. We believe that the PCT may be satisfactorily employed as a screening test in a clinical laboratory or blood bank.—*A. Balows.*

PORTNOY, Garson and Smith[4] were the first to report the use of plasma inactivated by a chemical anti-inhibitor rather than heat inactivated serum as the source of reagin in a serologic screening test for syphilis. This test, the Rapid Plasma Reagin test (RPR), has been widely used in certain areas requiring a rapid serologic procedure and it has been accepted as an approved serologic test for syphilis by the United States Public Health Service.[5]

Subsequent to the introduction of the RPR, Andujar and Mazurek[1] reported on a serologic screening test called the Plasmacrit (PCT) which is essentially a micro RPR. The PCT possesses certain refinements, is more exacting in the procedure to be followed and offers a more consistent degree of accuracy and reproducibility. Despite the fact that plasma has a higher reagin content than serum it cannot be used for serologic tests because of the presence of undefined flocculation inhibitors. The filtrate obtained from heated and coagulated plasma has been used and it will yield flocculation,[2] but this is not a procedure that lends itself to the routine work of the clinical laboratory.

Ecker, *et al.*,[3] showed that choline chloride is one of several organic salts that will effectively neutralize the flocculation inhibitory component of either serum or plasma; this inhibition is reversible as contrasted to the irreversible destructive action of heat.

In the development of the plasmacrit, Andujar and Mazurek tested almost three thousand serums and plasmas with a battery of accepted V.D. serologic tests and the new PCT. Their results clearly indicate that the PCT is highly sensitive and reasonably specific. This high degree of accuracy and the relative ease and simplicity of the PCT was particularly appealing to us in that it would provide us with a reliable screening test that would greatly expedite routine V.D. serology. We elected to evaluate the PCT by performing the VDRL on serum and the PCT on plasma of all patients referred to the laboratory for a VDRL.

Materials and Methods

A. A venipuncture was done on all individuals to be tested and the blood obtained was divided into two portions. One portion was added to a clean tube and allowed to clot. The second portion was added to a tube with an anticoagulant such as EDTA or oxalate.

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B. The VDRL was performed on all serums using the standard procedure[5] including reactive and nonreactive controls.

C. The plasmacrit was performed as follows:

1. Whole blood from the anticoagulant tube was drawn into a heparinized capillary tube as specified by Andujar and Mazurek and sealed at one end with Critoseal.†
2. The capillary tubes were then centrifuged for three minutes at 11,000 rpm. in a micro-hematocrit centrifuge.
3. Using a metric rule, three marks were made on the capillary tube starting at the free end of the plasma column; one mark at 20 mm., the second at 22, and the third at 26 mm. A small nick was made at the 22 mm. mark with a glass ampoule file.
4. The capillary tube was snapped at the 22 mm. nick and the plasma was placed in a ceramic ring on a standard VDRL glass slide, using a vaccine bulb to expel the plasma. Andujar and Mazurek very carefully determined that the length of the plasma column required to give the optimal plasma-antigen ratio was 22 mm. The maximal and minimal plasma column lengths allowable were 26 mm. and 20 mm., respectively. We found our method of marking the plasmacrit capillary tube at the above mentioned lengths and nicking at the 22 mm. mark gave us double assurance of obtaining the optimal volume of plasma.
5. With a tuberculin syringe and a 25 gauge no-bevel needle, previously calibrated to deliver 100 drops per ml., one drop of choline chloride antigen was added to each ceramic ring containing plasma. The choline chloride antigen emulsion was prepared according to the alternate method offered by Andujar and Mazurek; that is, regular VDRL slide emulsion was prepared as directed substituting 10 per cent choline chloride in physiologic saline in place of saline or buffered saline. We prepared a fresh batch of choline chloride antigen each day along with the daily batch of VDRL emulsion.
6. A plasma control was included with each run, using the plasma aspirated from VDRL reactive blood discarded by the blood bank. This control plasma is dispensed in 2 ml. amounts and frozen. One 2 ml. vial will last for four to seven days without loss of reactivity.
7. The slides were placed on the serologic rotator and rotated for four minutes.
8. The slides were read promptly using a microscope with 100 X magnification and reported in the same manner as the VDRL. The choline chloride antigen is somewhat coarser than the VDRL antigen but this presents no problem after a few practice runs.

† Manufactured by Biological Research, Inc., St. Louis, Mo. and distributed by Scientific Products Co., catalog number 52476.

Results

The slide VDRL and PCT were performed on serum and plasma obtained from 2,458 individuals. This group represents a good sampling of what is usually encountered in practically every clinical laboratory. Some were patients being examined by their physician for a specific complaint or for a routine examination, some were hospitalized patients with known or suspected syphilis, several were blood donors, and still others were complying with premarital, prenatal, or pre-employment regulations.

As previously stated, it was not our intent to determine the specificity or the degree of sensitivity of the PCT. This has already been established by Andujar and Mazurek. We were concerned primarily with evaluating the PCT as a screening test for routine use in a clinical laboratory by comparing it with the VDRL. Our data, tabulated

TABLE 1. *Comparison of Results from PCT and VDRL Tests for Syphilis*

VDRL	PCT	Number	Per cent
Nonreactive	Nonreactive	2,380	96.82
Weakly reactive	Weakly reactive	31	1.26
Reactive	Reactive	32	1.33
Nonreactive	Weakly reactive	3	.12
Nonreactive	Reactive	3	.12
Weakly reactive	Nonreactive	0	00
Weakly reactive	Reactive	8	.31
Reactive	Nonreactive	1	.04
Reactive	Weakly reactive	0	00
	Total	2,458	100

in Table 1, clearly indicate that the PCT yields results that are comparable with the slide VDRL test.

The same results were obtained with both tests on 2,443 individuals, *i.e.*, 2,380 were non-reactive, 31 were weakly reactive and 32 were reactive. This represents complete agreement between the VDRL and the PCT in 99.4 per cent of the total number tested. Different results were obtained with both tests on 15 specimens. Eight sera were weakly reactive by the VDRL test and plasma from the same patients gave a reactive PCT. In like manner, non-reactive VDRL results were obtained on sera from six patients while the plasma gave a weakly reactive PCT in three cases and a reactive PCT in three. The serum of each of these six patients was tested by the standard Kahn test and the Kolmer complement fixation test and were all non-reactive. The results were therefore reported as non-reactive. We ascribe this difference to the greater sensitivity of the PCT which, in our opinion, enhances the value of the PCT as a screening test.

In only one case was the VDRL reactive and the PCT non-reactive. Both tests were repeated on a second blood specimen and the same results were obtained. Kolmer complement fixation tests were anticomplementary and the patient refused to return to the laboratory so we could obtain some more blood. We are unable to offer an acceptable explanation for this except to note that the referring physician informed us that the patient was a "known case" and had been treated for some time without reversal of his serology. Andujar and Mazurek encountered a similar situation with one individual in their report and it is not at all uncommon to find in any laboratory an occasional disagreement of the results of two different serologic tests for syphilis performed on the same serum specimen.

Discussion

In our approach to the comparison of the VDRL with the PCT it was necessary for us to deviate from the protocol of Andujar and Mazurek. The PCT was devised as a screening test by them and the use of capillary blood from a finger prick was the recommended procedure of obtaining the blood for the test. However, the PCT can be performed on oxalated venous blood which we elected to use. We were reluctant to submit all the people tested to a venipuncture and a finger prick at the same time. In the actual application of the PCT in our laboratory, capillary blood is used unless other laboratory work requested on the same individual requires venous blood. We were also able to omit the centrifugation of the antigen emulsion and resuspension of the sediment in choline chloride as

recommended by the authors. Our antigen emulsions were quite antigenic when 10 per cent choline chloride was used as the diluent in place of the buffered physiologic saline used in preparing the standard VDRL antigen.

As our investigation progressed, several of the advantages and practical applications of the PCT as pointed out by Andujar and Mazurek were readily realized. The procedure itself speaks for the relative simplicity and ease of performance. We feel that our number of duplicate tests performed lends support to the sensitivity and specificity of the PCT as claimed by the originators. The Kentucky State Board of Health has endorsed the PCT as an approved procedure for V.D. testing for premarital, prenatal, pre-employment, prospective blood donors, and other instances where such a test is required.* With this approval, a rapid service is now available. For example, prospective blood donors can be screened prior to the actual drawing of the blood by venesection. Capillary blood can be obtained by a finger prick and the capillary tube placed in the micro-hematocrit centrifuge. Following centrifugation the hematocrit can be read if so desired or indicated and then the PCT test performed. During this interval the required physical examination and additional processing of the donor may be continued. Thus in 15 minutes a prospective donor can be processed and the blood bank can be reasonably assured that the blood will be acceptable. In the event the PCT gives a reactive or weakly reactive reading the donor is still available for venipuncture, at which time an ample supply of blood can be drawn for a battery of tests.

We are in complete accord with Andujar and Mazurek concerning the several advantages and practical applications of this test. In our hands the PCT was rapidly performed with a minimum of inconvenience and was equal to or better than the VDRL in sensitivity and specificity.

We also concur with the originators that the PCT is a reliable screening test when performed by competent laboratory technologists. All reactive or weakly reactive PCT results should be checked further with one or more of the standard tests for syphilis using serum before a final report is issued.

While the PCT as described by Andujar and Mazurek was designed to be performed on capillary blood obtained by a finger prick, we were able to obtain equally reliable results using venous blood collected by venipuncture and preserved with a suitable anticoagulant.

* The National Institutes of Health has endorsed the PCT as an acceptable test for syphilis provided it is approved by the state authorities.

Summary

We have performed the PCT and the VDRL simultaneously in 2,458 subjects. Our results indicate that the plasmacrit test of Andujar and Mazurek has proved to be a reliable adjunct to the VDRL serologic test for syphilis. We believe that the PCT may be satisfactorily employed as a rapid serologic screening test for syphilis. The application of this test to the routine V.D. serology testing in a clinical laboratory or blood bank has been discussed.

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Effects of Salt Concentration, *p*H, and Certain Ions on the Agglutination of Human Erythrocytes by Isohemagglutinins*

M. YOKOYAMA, M.D., J. S. FINLAYSON, PH.D.

From the Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Division of Biologics Standards, Bethesda 14, Maryland

The effect of molarity of NaCl, KCl, LiCl, tris(hydroxymethyl)aminomethane acetate, $CaCl_2$, $MgCl_2$, Na_2SO_4, K_2SO_4, and $MgSO_4$ solutions on the agglutination of human erythrocytes by isohemagglutinins of the ABO series has been studied, as has that of *p*H in "tris acetate" buffer. In all cases the maximum agglutination titer occurred at the isotonic or a slightly hypertonic concentration and was generally the same as that determined by diluting the given cells and serum with isotonic saline. Moreover, all salts exerted quantitatively similar effects on agglutination, the titer decreasing regularly as the molarity increased from 0.154-0.308 to 1.0. Apparent variations in the effects of different classes of salts were induced by expressing salt concentration as ionic strength or normality rather than molarity.

In tris acetate buffers of ionic strengths 0.154 (isotonic) and 0.308, the titer was constant in the *p*H range 5.5 to 7.5-8.0 and decreased at either extreme.

Introduction

DURING a study involving the screening of a large number of solutions of human serum proteins for isohemagglutinin activity, we became concerned with the possible effects of salt concentration and *p*H on our measurements of agglutination titer. While a search of the literature yielded many references to the action of electrolytes on bacterial and viral agglutination and to the agglutination of erythrocytes by salts or acid, very few papers were devoted to the effects of salt concentration or *p*H on the agglutination of red cells by serum agglutinins.[1, 3-5] Moreover, much of this work dealt with the agglutination of the cells of one animal species by the serum of another. The present investigation therefore treats the effects of the concentration of various salts, as well as *p*H, on the agglutination of human erythrocytes by isohemagglutinins of the ABO series.

Methods

Chemical and Serological: Various inorganic salts were used to prepare solutions ranging in ionic strength from 0.039 to 3.0. Tris (hydroxymethyl)aminomethane† and acetic acid were used to prepare a series of "tris acetate" buffers, *p*H 7.0, having this range of ionic strengths. Two series of tris acetate buffers, ionic strengths 0.154 and 0.308 respectively, were prepared so as to achieve a range of *p*H values between 4.0 and 9.0. This buffer was chosen because a wide *p*H range could be obtained using the same two ions.

These solutions were used to make 2 per cent suspensions of fresh red cells of group A_1 and group B, and to make serial dilutions of fresh group A, B, and O sera inactivated by heating at 56° C. for 30 minutes. Cells suspended in a given solution were mixed in a small test tube (10 mm. × 75 mm.) with the appropriate serum diluted with that same solution, one drop each of cell suspension and diluted serum being used. The tubes were agitated and allowed to stand at room temperature for 30 minutes. They were then centrifuged for 30 seconds at 500 rpm. and examined macroscopically for agglutination. The agglutina-

* Received for publication September 2, 1960; accepted November 25, 1960.

† Sigma 7-9, Sigma Chemical Company, St. Louis, Missouri.

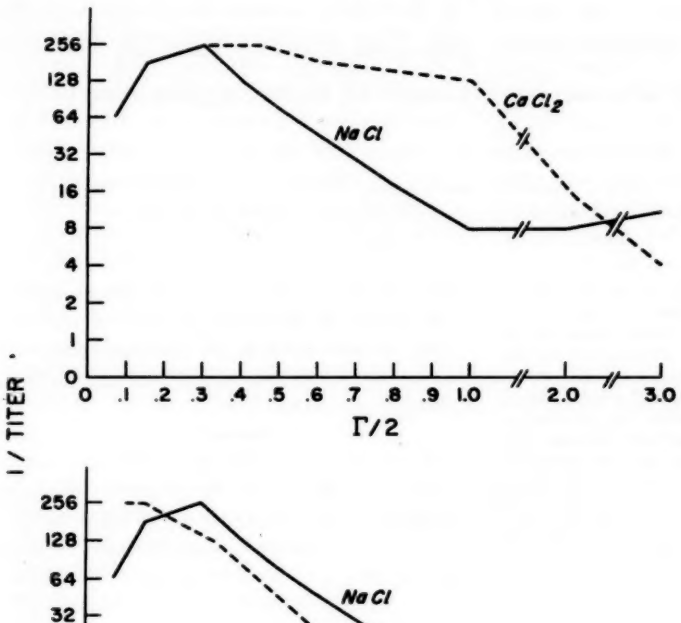


FIG. 1. Effect of expressing salt concentration as molarity or ionic strength. 1*A*. Titer versus ionic strength. 1*B*. Titer versus molarity.

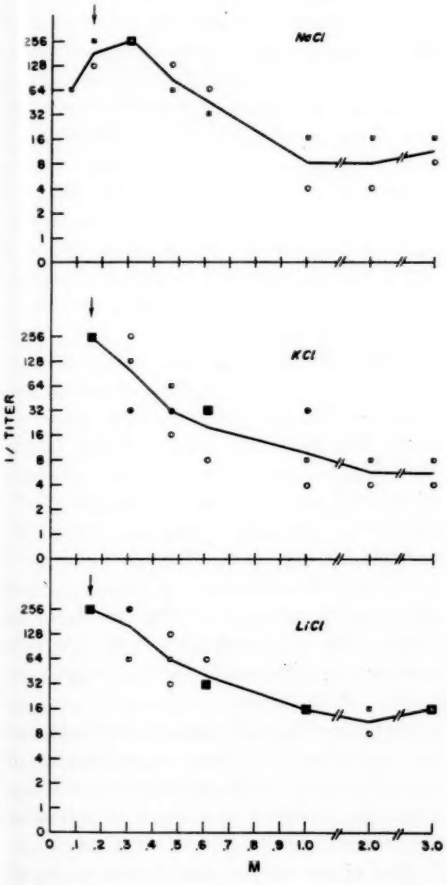
tion titer was determined as the highest dilution showing 1 (+) clumping of cells. Thus "doubtful" reactions and artifacts introduced by cells adhering to the tube walls did not affect the final titer.

Each salt solution, freshly prepared, was tested two to four times with different cells and sera. Experiments were also carried out in which the cells were diluted with isotonic saline. Because the results of these tests were qualitatively the same as those involving cells diluted with the experimental solutions, only the results of the latter have been presented.

Expression of Results: The results have been presented graphically, agglutination titer being plotted on a logarithmic scale *versus* *p*H or salt concentration on a linear scale. Since the sera used were of somewhat different titers, the results obtained with all sera have been adjusted so that the maximum titer of each replicate experiment received the value of 1:256. This procedure has permitted clearer presentation of the data and appears justified since, for any given salt, the curve maintained the same shape regardless of the absolute titer values.

When this study was undertaken it was

intended to prepare graphs of titer *versus* salt concentration expressed as ionic strength. The use of this procedure yielded at least two types of curves for different classes of salts. On the other hand, when titer was plotted against salt concentration expressed as *molarity*, very different results were obtained. Consider, for example, a comparison of the effects of NaCl and $CaCl_2$ on agglutination. Expressed as titer *versus*



FIGS. 2*A*, *B*, *C*. Effect of salt concentration on agglutination titer. Vertical arrows indicate the isotonic concentration. Each type of symbol (circle, dot, square, or triangle) denotes a single set of determinations. Use of multiple symbols or half-filled circles indicates that points coincided.

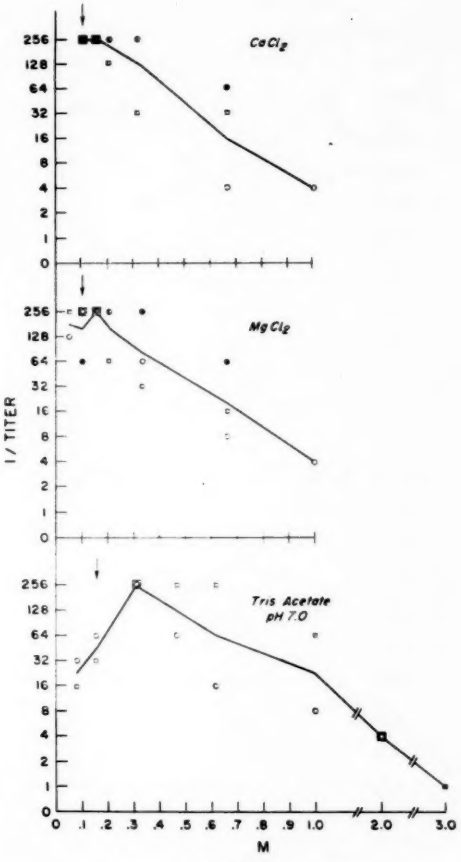


FIG. 2*B*.

ionic strength (Fig. 1A), these two salts showed widely differing curves of activity. When salt concentrations were expressed as molarity (Fig. 1B), the two curves were essentially identical within the variability of the method.†† Since molarity is the more accessible quantity, results have been reported as titer *versus* molarity; however, apparent differences induced by using ionic strength will be pointed out.

†† It may be pointed out that a 1 molar solution of a divalent salt such as $CaCl_2$ has a normality of 2 and an ionic strength of 3.

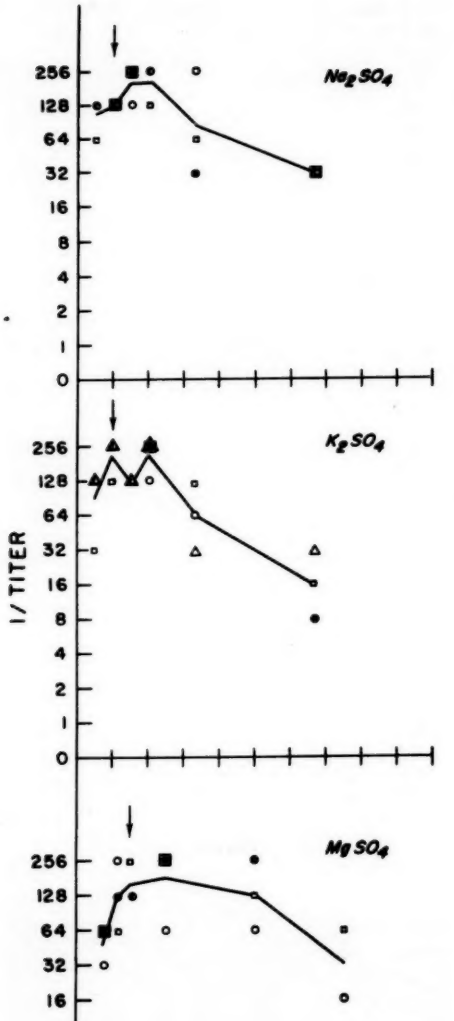


FIG. 2C.

Results

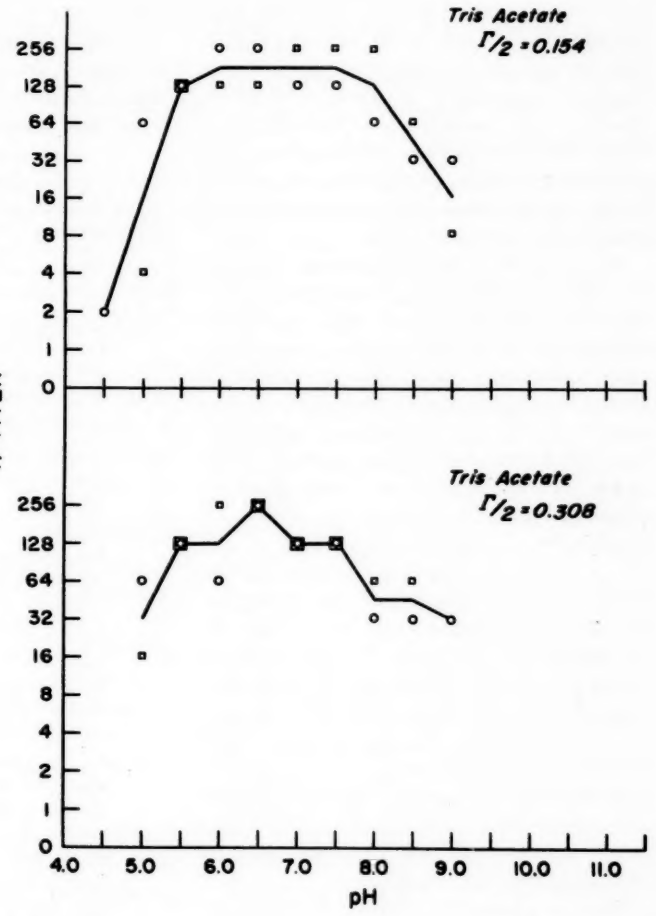
In all salt solutions the maximum agglutination titer occurred at the isotonic or a slightly hypertonic concentration (Fig. 2). The maximum titer was generally the same as that determined by diluting the given cells and serum with isotonic saline, though titer differences of one dilution in each direction were seen. As expected, the hypotonic solutions usually caused hemolysis, but in several of the solutions ($MgCl_2$, K_2SO_4, Na_2SO_4, $MgSO_4$, and tris acetate) at half isotonic strength the cells remained essentially intact and showed agglutination.

Within the limits of error of the method, all of the salts exerted quantitatively similar effects on agglutination, the titer decreasing regularly as the molarity was increased from 0.154-0.308 to 1.0 (Fig. 2). There was a suggestion that moderately high (0.3 M to 0.5 M) concentrations of NaCl, $MgSO_4$, and tris acetate lowered the titer less than did comparable concentrations of the other salts.

When the concentrations were expressed as ionic strength, $MgCl_2$, Na_2SO_4, and K_2SO_4 exhibited curves which were virtually identical with that of $CaCl_2$ in Fig. 1A. A curve based on the ionic strengths of $MgSO_4$ was very flat and displaced still farther to the right. Similar, though less exaggerated, apparent differences occurred when the concentrations of these salts were expressed as normality. The curves for the monovalent salts are, of course, the same whether the abscissa is ionic strength, normality, or molarity.

An interesting additional observation was the fact that in all cases some adhesion of red cells to the walls of the tubes occurred when the molarity was three to five times the isotonic concentration or greater.

The effects of *p*H are shown in Fig. 3. At ionic strengths of 0.154 (isotonic at *p*H 6.5) and 0.308, the titer was constant in the *p*H range 5.5 to 7.5-8.0. At either extreme the titer decreased.



IG. 3. Effect of *p*H on agglutination titer. Each type of symbol (circle or square) denotes a single set of determinations.

Discussion

The present findings seem to be in agreement with that information which is available. Sai,[5] in a two-sentence abstract which did not indicate the species of either the cells or serum, reported the optimal *p*H range as 4.31 to 8.59, and Bogomasova found that, of a series of NaCl solutions, the 2 per cent NaCl gave the maximum acceleration of agglutination rate.[1] Forssman, *et al.*, generally employed a system involving sheep, bovine, pig, or occasionally human cells suspended in the experimental salt solution, tested against aged specific rabbit antisera, presumably diluted with isotonic saline. However, they also stated that agglutination of human cells by "isolysins" was weaker when the cells were suspended in 3 per cent NaCl than in 1 per cent, and was still weaker in 5 per cent NaCl.[3] Finally, Landsteiner and Welecki, using a test system consisting of the given salt solution, inactivated bovine serum and washed horse erythrocytes, found that raising the normality of the salt from 0.125 to 0.5 greatly decreased the agglutination in-

tensity in the case of most salts and that raising it to 1.0 did so in all cases.[4] It is interesting that the inorganic salts which required a normality of 1.0 rather than 0.5 to exert a noticeable effect were divalent. Thus, had these workers expressed salt concentration in terms of molarity, the actions of the various electrolytes would have appeared even more uniform.

Many workers have considered the role of surface charges and cell:medium potential differences in bacterial agglutination as a basis for the action of salts and acid in these systems.[2, 6] More recent studies reviewed by Boyd[2] have cast doubt on the role of altered surface charges in agglutination. One can suggest that increasing salt concentrations might exert their effect by altering the agglutinogen, by causing a non-specific change in the surface of the erythrocyte, by altering the agglutinin, by shifting the equilibrium of the agglutinogen-agglutinin reaction, or by salting out the agglutinin. Only the last possibility is rendered unlikely by the present work. For example Na_2SO_4, a common protein precipitant, depressed the agglutination titer appreciably at a concentration of 0.33 M, whereas 0.95 M is usually used to precipitate γ-globulins from serum. Moreover, strong solutions of NaCl can be used to solubilize absorbed antibodies.

From a practical viewpoint, it appears that a moderate amount of isohemagglutinin activity dissolved in a medium unfavorable for agglutination can be detected if isotonic saline (or, perhaps, buffered isotonic saline) is used to suspend the erythrocytes and to make serial dilutions.

Acknowledgment

The authors are grateful to Mrs. Virginia Fine for diligent technical assistance.

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A New Antibody, Anti-Ku (anti-Peltz), in the Kell Blood-Group System*

PATRICIA A. CORCORAN, FRED H. ALLEN, JR., M.D., MARION LEWIS, BRUCE CHOWN, M.D.
From the Blood Grouping Laboratory, Boston and the Rh Laboratory, Winnipeg

Analysis was made of isoantibody in the serum of Mrs. Peltz, who is of the very rare Kell type K−,k−,Kp(a−b−). Successive adsorptions and elutions were done using three bloods selected in such a way that the eluate finally obtained could contain no previously known blood-group antibody. It agglutinated all of 525 bloods chosen at random, and failed to agglutinate only very rare bloods that were of highly unusual Kell types.

TWO UNRELATED families have been reported in which the Kell phenotype K (Kell) negative, k (Cellano) negative, Kp^a (Penney) negative, Kp^b (Rautenberg) negative occurred.[2, 4] The propositus (Mrs. Peltz) of the first family had in her serum antibodies that caused hemolytic disease in her fetus. The serum has now been more thoroughly analyzed; it contains an antibody that appears to be a new antibody in the Kell system, here designated anti-Ku. The antibody seems to detect an antigenic factor in the Kell system of nearly universal occurrence in human red cells.

Methods

Since Mrs. Peltz's serum agglutinated nearly all bloods, and since she had the unusual phenotype K−, k−, Kp (a−b−), it was considered possible that there was a new antibody in the serum comparable to the anti-Hr_o believed to be present in the serum of immunized persons of Rh phenotype C−, c−, E−, e−.[6] Such a serum is nearly impossible to characterize satisfactorily because of the rarity of bloods that it does not agglutinate. The plan was, therefore, to eliminate all known antibodies by adsorption and elution with selected red cells, using a method successfully tried by Hackel for a slightly different purpose.[3]

The Peltz serum was first adsorbed onto cells of type K−, k+, Kp (a+b−), then eluted; this eluate was then adsorbed onto cells of type K+, k−, Kp (a−b+) and again eluted; this second eluate was then adsorbed onto cells of type K−, k+, Kp (a−b+) and again eluted; this final eluate was designated "triple eluate." The three bloods used for the various adsorptions were so chosen as to eliminate the possibility of most of the known antibodies, including previously known antibodies of the Kell system, being present in the final eluate. The absence, in the triple eluate, of antibodies against certain very common blood-group antigens is demonstrated in Table 1. By these maneuvers the antibody in the triple eluate was shown to be none of the known specific antibodies, nor any mixture of known antibodies.

Adsorption was for 60 minutes at 37 C. Elution was done by the method of Weiner,[5] except that 6 per cent bovine albumin was used instead of serum or saline to take up the eluted antibody. Cells were washed three times in large quantities of saline before elution, to be sure that no antibody was carried over except that specifically attached to the cells. All testing was by the indirect antiglobulin method.

Results

Table 1 gives the results of tests with the triple eluate against certain highly selected bloods, showing positive reactions with a K+ homozygote (R.R.), a Kp (a+) homo-

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Supported by Grant H 2405 from the National Institutes of Health.

TABLE 1. *Tests of Triple Eluate from Peltz Serum Against Bloods of Selected Types*

	Triple eluate	H	U	K	k	Kp^a	Kp^b	Tj^a	Vel	Yt
Peltz	0			0	0	0	0			
Kanto	0	+	+	0	0	0	0	+	+	+
G.R.	+			0	+	+	0			
R.R.	+			+	0	0	+			
L.S.	+			0	+	0	+			
McLeod*	0			0	var.	0	var.			

Triple eluate prepared by successive absorption from the three donors described in the text.
Failure of the triple eluate to agglutinate the blood of Mrs. K. shows that it does not contain anti-H, -U, -Tj^a, -Vel, -I, or -Yt^a.
*McLeod represents a new and not completely classified Kell phenotype. He gives some of the reactions (but not all) expected of a k (Cellano)-positive, and of a Kp^b (Rautenberg)-positive. var. = variant

zygote (G.R.), and a K−, Kp(a−) homozygote (L.S.). Negative reactions were obtained with the bloods of Mrs. Peltz herself and Mrs. Kanto who, though unrelated, apparently is of the same rare Kell type as Mrs. Peltz.[4] The triple eluate failed also to agglutinate the red cells of Mr. McLeod: his Kell phenotype is different from any previously reported, and study of his blood is the subject of a separate report.[1] A fourth negative reactor was the sister of Mrs. Peltz, who has the same rare Kell phenotype.[2]

Further tests of triple eluate were done with 525 random bloods, of which 33 were from Negroes. All these tests were positive.

Discussion

That the Peltz antibody belongs to the Kell system is clear from her own unusual Kell phenotype and that of the other three persons whose red cells are not agglutinated by it. For this reason a name beginning with K was chosen. The "u" of "anti-Ku" was used because the antibody appeared to detect an antigenic factor of nearly universal occurrence in human red cells.

There appear to be two possible explanations of the Peltz antibody (triple eluate). It may be an antibody that detects a new and nearly universal Kell factor, as postulated above, or it may be an antibody with two or more specificities on the same molecule (such as anti-Kk, or anti-Kp^aKp^b, or some other like combination). We prefer the first explanation as a working hypothesis because it can be put to the test whereas the second cannot, so far as we can see.

Table 2 gives the classification of the first four Kell genes, as demonstrated by the reactions of five Kell-system antibodies with four homozygotes. These reactions were confirmed by tests with a number of other bloods of genotypes K^bK^b, k^bk^b, and k^ak^a.

The technic of multiple adsorptions and elutions is one that is useful in dealing with an antibody such as this that agglutinates almost all bloods. It is otherwise

TABLE 2. *Characterization of the First Four Genes of the Kell System*

Genes	Antigenic factors produced				
	K	k	Kp^a	Kp^b	Ku
K^b	+	0	0	+	+
k^b	0	+	0	+	+
k^a	0	weak	+	0	+
K^o	0	0	0	0	0

most difficult to be sure that other antibodies, perhaps of several different specificities, are not adding confusion to the problem of identification.

Summary

A new antibody (anti-Ku) in the Kell system is described. It is believed to be a specific antibody, detecting an antigenic factor of very high frequency. The antibody was produced by a woman of the very rare type K−, k−, Kp (a−b−).

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Technical Note

Preparation of a Stable Bromelin Solution*

MARGERY P. GRAY, PH.D.

From the Department of Medicine, University of Wisconsin Medical School Madison, Wisconsin

ACTIVE but unstable bromelin suspensions can be prepared by mixing crude bromelin** powder with physiological saline or phosphate buffer.[1, 2] Stable solutions can be purchased but the cost is much greater than for a comparable amount of crude bromelin.

In investigating the effect of divalent ions on bromelin activity, it was found that addition of EDTA† (ethylene-diamine-tetra-acetic acid, di-sodium salt) produced a stabilizing effect on enzyme activity.

Materials and Methods

To 20.0 mg. of crude bromelin, add 20.0 mg. of EDTA and bring to 10.0 ml. with physiological saline. The opalescent suspension can be used immediately if desired since the insoluble material does not interfere. A clear solution can best be obtained by allowing the sediment to settle overnight at 4 C. and decanting the supernatant. If stored at 4 C., bromelin activity will remain stable for at least one month.

Results and Discussion

EDTA-bromelin can be used for the detection of many immune and naturally occurring antibodies but it must be kept constantly in mind that some antibodies, especially M, N, and Fy^a, will not be detected.[1] The reactions of cold and warm "auto-agglutinins" are greatly enhanced by addition of bromelin. In cases of hypoglobulinemia, bromelin increases the avidity and titer of anti-A and/or B to a level that ensures a reliable serum confirmation of the ABO type. The activity of plant lectins is also intensified by addition of bromelin.

Summary

A method is described for the preparation of a stabilized solution of bromelin.

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** Wallerstein Laboratories, New York.

† Hartman-Leddon, No. 5693.

Short Communication

Effect of Prednisolone on Incompatible Blood Transfusions*

WILMER C. HEWITT, JR., CAPTAIN, MC, MUNSEY WHEBY, CAPTAIN, MC, WILLIAM H. CROSBY, COLONEL, MC

From the Department of Hematology, Walter Reed Army Institute of Research Washington, D. C.

In control experiments small volumes of incompatible, chromium^{51} tagged red cells were given to recipients. Several days later the studies were repeated 30 minutes after the intravenous administration of prednisolone. Prednisolone was found completely ineffective in prolonging the survival of small amounts of red cells in the presence of incompatible antibodies of the ABO and Rh systems.

DURING the 1959 meeting of the American Medical Association, a scientific exhibit by Offenkrantz, Blaustein and Babcock presented data on the use of intravenous prednisolone with blood transfusions in which difficulty in crossmatching existed.[8] They indicated that this drug was effective in preventing hemolytic reactions associated with minor-side incompatibilities. In none of the cases reported, however, was a red cell survival time determined. Based on this work, the Schering Corporation has included in the brochure which accompanies each unit of prednisolone (Meticortelone®) that the drug is indicated in "cases which involve special situations with difficult crossmatch as well as known isoagglutinin."[1] Jandl and Tomlinson[6] noted that cortisone had no effect on the rate of destruction of incompatible red cells given to human volunteers. Because of the obvious importance of clarifying this matter, the present study was undertaken.

Methods

Recipients for the ABO incompatible transfusions were laboratory and hospital personnel demonstrating no hematological abnormalities. In the studies of Rh incompatibility, one patient who was recovering from an hemolytic transfusion reaction due to an anti-c was used. No renal involvement was present. The second patient had previously undergone a splenectomy for autoimmune hemolytic anemia associated with disseminated lupus erythematosus. At the time of study he had a weak anti-E. The blood type of each recipient and the type of blood transfused is listed in Table 1.

Preparation of the blood, including tagging with $Na_2Cr^{51}O_4$, followed previously described methods.[4, 5] Survival time is expressed as half life of the radioactivity in the red cells.

After base line blood counts and plasma hemoglobin levels were obtained, the subject was given a rapid intravenous transfusion of 1 to 3 ml. of incompatible, chromium^{51} tagged cells. Blood samples were then taken at brief intervals for determination of the red cell and plasma radioactivity, plasma hemoglobin, white blood cell and platelet counts. Several days later, using blood from the same donor, an identical experiment was performed with the exception that 90 mg. of prednisolone were given intravenously 30 minutes prior to transfusion. An additional 10 mg. were added to the cell suspension to be administered.

Plasma hemoglobin was determined by the method of Crosby and Furth.[3] White blood cell counts and platelet counts were performed using standard technics.[1] Red cell and plasma chromium^{51} activity were determined in a well-type scintillation counter.

* Received for publication November 10, 1960; accepted December 30, 1960.

TABLE 1.

Recipient	Donor cell type	Prednisolone amount and route	Control survival time (Cr^{51} t/2)	Survival time with prednisolone (Cr^{51} t/2)	Remarks
A Rh+	B Rh+	100 mg. P.O.	< 2 min.	< 2 min.	
A Rh+	B Rh+	100 mg. P.O.	2 min.	2 min.	
A Rh+	B Rh+	100 mg. I.V.	5 min.	3 min.	
A Rh+	B Rh+	100 mg. I.V.	< 2 min.	< 2 min.	
A Rh+	B Rh+	100 mg. I.V.	< 2 min.	< 2 min.	
A Rh+	B Rh+	100 mg. I.V.	< 2 min.	< 2 min.	
O Rh+	A Rh+	100 mg. I.V.	–	< 2 min.	
B Rh+	A Rh+	100 mg. I.V.	–	< 2 min.	
A $\frac{CDe}{cde}$ Anti-E	O $\frac{cDE}{cDE}$	100 mg. I.V.	2½ hrs.	6½ hrs.	Autoimmune hemolytic anemia post splenectomy
A $\frac{CDe}{CDe}$ Anti-c	A_1 $\frac{cDE}{cDE}$	100 mg. I.V.	4 hrs.	1 hr.	Subsiding incompatible transfusion reaction

Results

The chromium^{51} red cell survival times are shown in Table 1. As can be seen there was no significant difference in the survival of the incompatible cells when prednisolone was used. The pattern of destruction regardless of prednisolone was as described previously.[5, 7] The graphic results of an ABO incompatible transfusion experiment are shown in Figure 1. Although none of the patients developed symptoms during the experiments, four to six minutes after receiving the incompatible ABO cells they showed a 25 to 70 per cent decrease in white cell count with or without prednisolone. A minimal fall in platelet count occurred at the same time in five of the ABO group. The greatest decrease was 20 per cent of the control level. In the ABO experiments in which hemolysis was immediate the plasma hemoglobin rose to levels expected with total intravascular hemolysis. In two of this group with half-survival times of two and three minutes, the maximum level was below that predicted thus indicating some extravascular destruction.

The patients with Rh incompatibilities did not demonstrate significant changes in

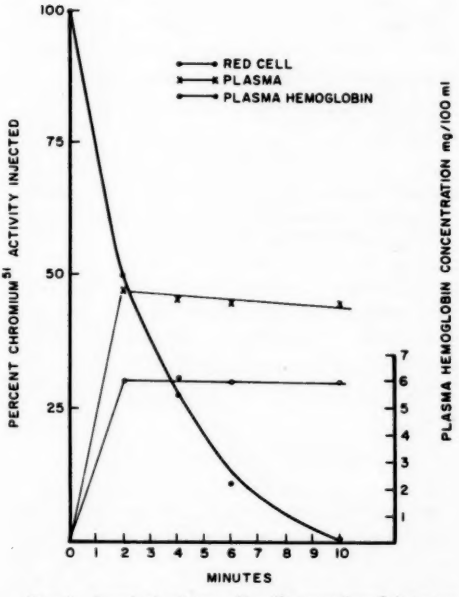

FIG. 1. Survival of type B cells transfused into an A subject is illustrated. The t/2 of the incompatible cells is 2 minutes. A prompt rise in plasma radioactivity and plasma hemoglobin indicated intravascular hemolysis. An identical pattern was seen when prednisolone was given.

the white cell or platelet counts. The subject without a spleen showed no rise in plasma hemoglobin while the other had a small increase approximately one hour after injection.

The use of prednisolone did not alter these findings.

Discussion

The rates of hemolysis in these experiments are similar to those reported by others.[5, 7] Furthermore, our results indicate that prednisolone is not effective in preventing the rapid destruction of transfused incompatible red blood cells. The drug, by virtue of its antipyretic action, may prevent the fever and chills associated with hemolytic or nonspecific transfusion reactions, but it is completely ineffective in prolonging survival of small amounts of incompatible cells. The most important conclusion from this study is apparent and serves to accentuate the recent editorial by Chaplin and Swisher.[2] There is no substitute for meticulous crossmatching of blood and when difficulty is encountered, one cannot rely on steroids to prevent a hemolytic reaction.

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TECHNOLOGISTS' SECTION

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New York, New York

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Chicago, Illinois

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Salt Lake City, Utah

MARION V. WALLER, PH.D.
Richmond, Virginia

Leukoagglutinin Studies as a Routine Procedure in Blood Banking*

ELEANOR HOWELL, B.A., BB (ASCP), BARBARA TAYLOR, M.L.T.

Irwin Memorial Blood Bank of the San Francisco Medical Society
San Francisco, California

For the past four years, the method presented here for the detection of leukoagglutinins has been successfully integrated into the routine procedures of a community blood bank. Patients' sera are tested against leukocytes from 12 Group O donors. White cell suspensions are prepared from defibrinated blood using 4 per cent polyvinylpyrrolidone (PVP) as a sedimenting agent. Tests have been performed on 276 patients, among whom 89 (32 per cent of the total) were found to possess leukoagglutinins. Of the 89 individuals in whose sera leukoagglutinins were demonstrated, 85 had a reported history of febrile transfusion reactions, frequently accompanied by chills, occasionally with urticaria. These 85 patients made up 57 per cent of 150 cases who had been reported as experiencing nonhemolytic febrile reactions shown by several workers to occur in patients with leukoagglutinins.

Also described are methods used for the preparation of leukocyte-poor units requested for patients whose sera have been shown to contain leukoagglutinins.

IN EVERY transfusion service, there have always been a number of unexplained nonhemolytic transfusion reactions characterized by chills and fever in varying degrees of severity. Such febrile nonhemolytic reactions have been shown by several workers to occur in individuals in whose sera leukoagglutinins can be demonstrated.[2, 5, 7-9, 11]

It has also been shown that, for patients with leukoagglutinins, reactions of this type can be prevented by the administration of units of blood from which the buffy coat has been removed.[2, 4, 9, 10]

Technics for detecting leukoagglutinins and for preparing leukocyte-poor units are practical procedures of real value in treating patients who have suffered such reactions. The present report will describe the methods used routinely in a community blood bank, summarize the results obtained over a three-year period and indicate the rate of increase in the number of leukoagglutinin determinations and in the utilization of leukocyte-poor units in the area served.

Materials and Method

The method used for three years in this laboratory was described by Payne.[8] For convenience in blood bank practice, the procedure has been modified to test each patient against the leukocytes of 12 Group O donors drawn at random from our donor population.

Preparation of Sera: Complete studies require approximately 20 ml. of clotted blood from the patient. The serum is removed imme-

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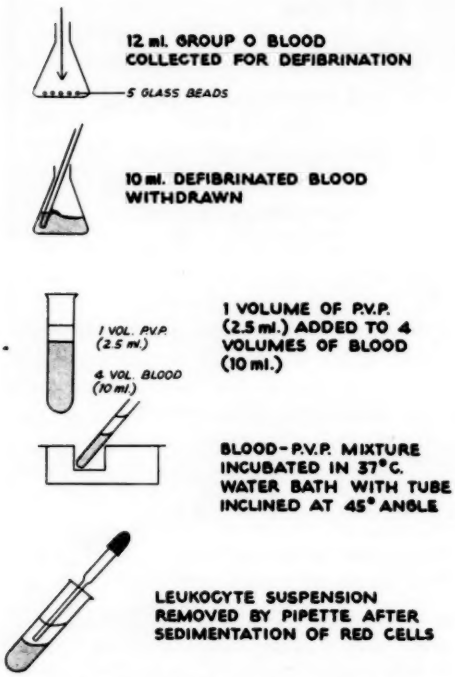
12 ml. GROUP O BLOOD
COLLECTED FOR DEFIBRINATION
5 GLASS BEADS
10 ml. DEFIBRINATED BLOOD
WITHDRAWN
1 VOL. P.V.P. (2.5 ml.)
4 VOL. BLOOD (10 ml.)
1 VOLUME OF P.V.P. (2.5 ml.) ADDED TO 4 VOLUMES OF BLOOD (10 ml.)
BLOOD-P.V.P. MIXTURE INCUBATED IN 37°C. WATER BATH WITH TUBE INCLINED AT 45° ANGLE
LEUKOCYTE SUSPENSION REMOVED BY PIPETTE AFTER SEDIMENTATION OF RED CELLS

FIG. 1. Preparation of leukocyte suspensions.

diately after the sample is received and aliquots are frozen, with no apparent loss of leukoagglutinin activity. All specimens of patients' sera are first tested for atypical erythrocyte antibodies capable of causing hemolytic reactions. When erythrocyte antibodies are present, to avoid the phenomenon of "mixed agglutination,"[1, 3, 10] donors must be employed whose red cells lack the corresponding antigens. When such donors are not available, the patient's serum must be absorbed until a Coombs test is negative against the donor's erythrocytes.

Time Required: To complete the tests within six to eight hours of drawing of blood for leukocyte suspensions, a maximum of six patients' sera may be tested against a panel of six donors. Each patient's serum is tested on at least two separate occasions. Tests are done once or twice a week depending on the number of sera to be tested.

Preparation of PVP: Four per cent polyvinylpyrrolidone (PVP),** used to enhance sedimentation of red cells, is prepared by dissolving 4 Gm. of PVP in 100 ml. of physiological saline. The solution is adjusted to between *p*H 6.8 and *p*H 7.2 with 0.1 N NaOH.

The method for preparation of leukocyte suspensions, diagrammed in Figure 1, follows:

1. For each leukocyte suspension, 12 ml. of blood from a group O donor are collected in a 50 cc. Erlenmeyer flask containing five glass beads and defibrinated by rotating gently for 10-15 minutes.
2. 10 ml. of defibrinated donor's blood are pipetted into a test tube measuring 1.8 cm. by 15 cm.
3. 2.5 ml. of PVP solution are added and mixed well with the blood.
4. Blood-PVP mixture is placed in a 37 C. water bath, with the tube at a 45 degree angle for 45-60 minutes, to obtain a well-defined supernatant containing a minimum of erythrocytes and a maximum of leukocytes.

Erythrocytes will sediment more rapidly than leukocytes, and at this step, the tube must be carefully and frequently observed, as the length of time required depends partly on the erythrocyte sedimentation rate of the donor. If the mixture is allowed to stand too long, leukocytes may also settle.

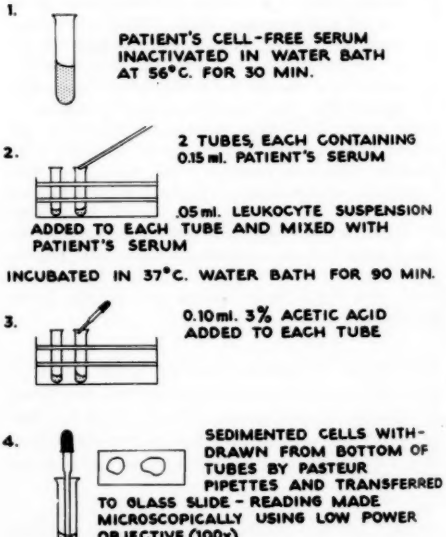
1.
PATIENT'S CELL-FREE SERUM INACTIVATED IN WATER BATH AT 56°C. FOR 30 MIN.
2.
2 TUBES, EACH CONTAINING 0.15 ml. PATIENT'S SERUM
.05 ml. LEUKOCYTE SUSPENSION ADDED TO EACH TUBE AND MIXED WITH PATIENT'S SERUM
INCUBATED IN 37°C. WATER BATH FOR 90 MIN.
3.
0.10 ml. 3% ACETIC ACID ADDED TO EACH TUBE
4.
SEDIMENTED CELLS WITHDRAWN FROM BOTTOM OF TUBES BY PASTEUR PIPETTES AND TRANSFERRED TO GLASS SLIDE - READING MADE MICROSCOPICALLY USING LOW POWER OBJECTIVE (100x)

FIG. 2. Test procedure for leukoagglutinins.

** Plasdone C (mean molecular weight 40,000) is supplied by Antara Chemicals, Sales Division of General Aniline and Film Corporation.

5. The supernatant is drawn off into another tube and leukocytes counted. A total leukocyte count of 3,000-5,000 wbc/cu. mm. is required. If the leukocyte count is above 5,000 per cu. mm., the suspension is diluted with normal serum.

Leukocyte suspensions must be used as soon as possible after preparation. They tend to clump spontaneously upon standing.

The test procedure for leukoagglutinins, illustrated in Figure 2, consists of the following steps:

1. Patient's serum is heated 30 min. at 56 C. to inactivate thermolabile inhibitors of leukoagglutination.
2. The test proper is set up in duplicate, each tube containing 0.15 ml. of patient's serum to which 0.05 ml. of leukocyte suspension is added.
3. The serum-leukocyte suspension mixtures are incubated 75-90 minutes at 37 C.
4. 0.1 ml. of 3 per cent acetic acid is added to each tube to lyse residual red cells; the mixture is allowed to stand 30 seconds to complete hemolysis.
5. With a separate Pasteur pipette for each tube, the sedimented cells are withdrawn from the bottom of the tubes and transferred to a glass slide. Centrifugation is not necessary and tends to produce false agglutination.
6. Slides are read microscopically under low power (100× magnification).

A serum known to contain leukoagglutinins and a normal serum control are set up with each leukocyte suspension. The latter is used as a check for non-specific clumping of white cells. The positive control proves the efficacy of the test-materials employed.

Figure 3 is a diagrammatic representation of negative, doubtful and positive results. Doubtful results may be due to foreign particles in serum or tube, presence of red cell rouleaux, incompletely lysed red cells. As the slides are read, results are recorded on forms which include the patients' names, blood groups, Rh types, diagnoses, erythrocyte antibodies when present, the blood numbers of the donor panel and the reaction of each patient's serum with each donor.

Reports give the number of donors whose cells were agglutinated as well as the number of donors whose cells were not agglutinated.

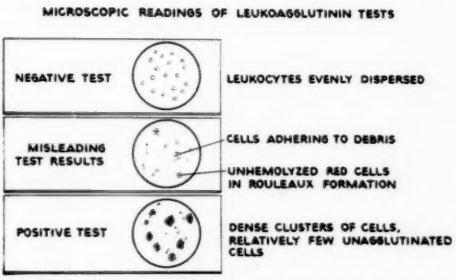
MICROSCOPIC READINGS OF LEUKOAGGLUTININ TESTS
NEGATIVE TEST
LEUKOCYTES EVENLY DISPERSED
MISLEADING TEST RESULTS
CELLS ADHERING TO DEBRIS
UNHEMOLYZED RED CELLS IN ROULEAUX FORMATION
POSITIVE TEST
DENSE CLUSTERS OF CELLS, RELATIVELY FEW UNAGGLUTINATED CELLS

FIG. 3. Diagrammatic representation of results of leukoagglutinin tests.

Test results are reported in this way because the sera of patients with leukoagglutinins do not react with all donors' cells.[4, 9, 11] For example, of 89 patients whom we have reported as giving positive results, 29 reacted with the leukocytes of all donors against which they were tested; the remaining 60 agglutinated the leukocytes of varying numbers of donors. Such reports may give the physician a rough guide to the frequency of febrile reactions which may be expected with whole blood.

Results of Tests for Leukoagglutinins

Within a three-year period, a total of 276 patients were tested for leukoagglutinins in this laboratory. Figure 4 indicates the rate of increase in requests for these tests. Eighty-nine, or 32 per cent of those tested, were found to possess leukoagglutinins in their sera. These total figures represent sera referred from a large area and a number of different services, with no real basis of selection. The 14 patients tested in the last six months of 1957 were selected because of non-hemolytic febrile reactions. Eight (57 per cent) of these cases had demonstrable leukoagglutinins in their sera. In 1958, 1959 and 1960, when tests for leukoagglutinins were performed as routine procedures on sera referred because of transfusion reactions of all types, many of the patients tested were found to have had non-pyrogenic allergic reactions or symptoms later shown not to be due to the administration of blood.

Of the 89 patients found to have leukoagglutinins, 81 had suffered non-hemolytic febrile reactions with no symptoms reported other than chills and/or fever, four had chills and fever and urticaria, and interestingly, four were reported to have had urticaria only. Of the 187 individuals in whom leukoagglutinins were not demonstrated, 59 had been reported as having experienced chills and fever, six as hav-

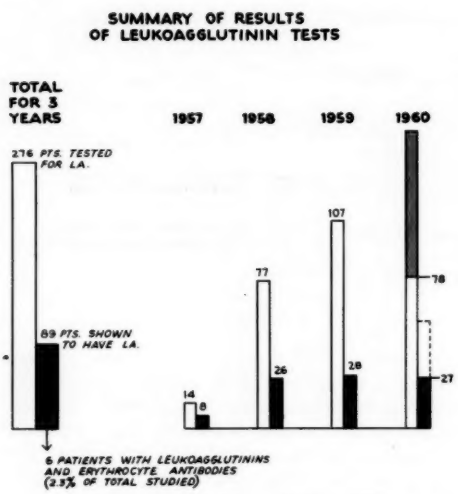

FIG. 4. Results of leukoagglutinin testing over a three-year period.

ing had chills and fever with urticaria. Of the remaining 122, 56 had been reported as having urticarial reactions, two had "wheezing," seven had complained of symptoms of dizziness, four of nausea, two of chills and dizziness, one of fever with slight pulmonary reaction. One had jaundice with fever, two had hematuria and 11 had complained of dyspnea. With 36 patients, no symptoms were stated and no adequate history obtained.

If the percentage of leukoagglutinins detected in this laboratory is calculated only on the basis of 150 patients for whom characteristic chill and fever reactions were reported, 85, or 57 per cent were shown to have developed leukoagglutinins, a figure corresponding surprisingly closely to that found in the 14 selected cases studied in 1957.

Of the 276 patients tested, 97 were men of whom 20 (approximately 20 per cent) possessed leukoagglutinins in their sera. One hundred and seventy-nine were women, with 69 (38 per cent) showing the presence of leukoagglutinins. Among the women, there were a few who suffered febrile reactions after receiving only one or two units of blood, suggesting as Payne and Rolfs[10] have reported, immunization by pregnancy.

Methods for Preparation of Leukocyte-Poor Units

For patients in whose sera leukoagglutinins have been demonstrated, requests are then made for leukocyte-poor units. Beginning in 1956, leukocyte-poor units were prepared in bottles containing 480 ml. of freshly drawn blood plus 120 ml. of Acid Citrate Dextrose, Solution B.[9] These were centrifuged in the cold for one hour at approximately 2,000 g. After centrifugation the plasma and buffy coat were removed, the remaining packed red cells were resuspended in sufficient physiological saline to restore the original volume, thoroughly mixed, and recentrifuged. The residual buffy coat was then removed with the supernatant saline. This method, used for three years, was very effective in the removal of leukocytes, consistently resulting in counts of less than 200 per cu. mm. However, it entailed at least 25 per cent loss of red cells with the aspiration of the buffy coat and had the disadvantage of entering the unit three or four times during processing.

The dextran sedimentation method[4, 7] was used to prepare 22 units for three special cases. No ill effects were encountered with this very limited trial. This method was found excellent for removal of leukocytes and resulted in very little loss of red cells, but also has the disadvantage of entering the units several times.

As the demand for leukocyte-poor units increased, a method was sought which would eliminate potential problems of sterility and save time in preparation. Since July 1959, leukocyte-poor units have been prepared in closed system plastic bags* with an integral donor tube and side pouch, containing blood drawn not more than 18 hours before processing is begun.[2, 4] The plastic bags containing 500 ml. of blood plus 75 ml. of Acid Citrate Dextrose, Solution A, are spun in an inverted

* Fenwal JD-2.

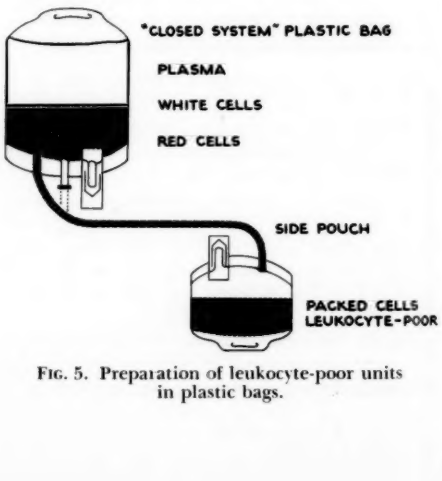

FIG. 5. Preparation of leukocyte-poor units in plastic bags.

position for one hour in a refrigerated centrifuge at 2,000 g. The bags are then suspended with great care not to disturb the buffy coat and the red cells are allowed to flow by gravity to the attached pack (Fig. 5). Approximately 175 Gm. of packed red cells (75 to 80 per cent of the red cell mass) can be transferred in this way without disturbing the leukocyte layer.

Samples are taken for leukocyte counts after the desired amount of red cells has been transferred. The tubing is clamped, leaving a considerable length of tubing on the side pouch. To make sure that the blood in the tube will represent a true sample of the blood in the bag, the contents of the tubing are "stripped" into the pack, mixed with its contents and the tube allowed to fill again. This process is repeated two or three times, before the tubing is tied and cut and a sample taken for leukocyte-count.

A unit of blood is not dispensed as leukocyte-poor unless the actual count is below 1,500 leukocytes per cu. mm. A great majority (81 per cent) of our preparations show leukocyte counts below 1,500 per cu. mm. In surveying the leukocyte counts made on 1,020 units prepared, it was found that they ranged from 140 leukocytes per cu. mm. to 3,700 leukocytes per cu. mm., with a mean of 1,090. 54 per cent of the counts were below 1,000 per cu. mm., 38 per cent below 700.

Utilization of Leukocyte-Poor Units: As the physicians in this area have become aware of the value and availability of special leukocyte-poor preparations for patients with leukoagglutinins in their sera, requests for such units have increased rapidly, from 38 units in 1956 to 429 prepared in 1959. It is expected that the number dispensed in 1960 will exceed 500. Figure 6 indicates that the rate of increase was most marked between January 1, 1958 and January 1, 1959. The total figure of 1,020 within a four-year period represents leukocyte-poor units administered to 60 patients in 21 hospitals.

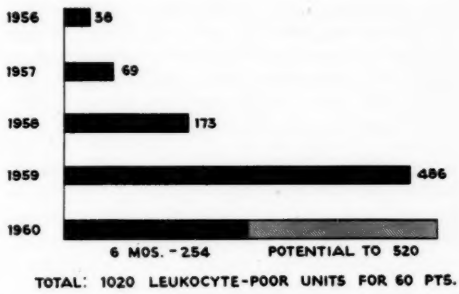

FIG. 6. Increase in number of leukocyte-poor units prepared per year.

Table 1 shows the number of leukocyte-poor units utilized by a series of patients who required unusually large numbers of transfusions. These were administered over periods varying from four weeks to three years. It should be noted that patients of relatively rare blood groups may require large numbers of leukocyte-poor units for which special donors must be called.

Discussion

Tests for leukoagglutinins have become practical procedures of value in determining the cause of febrile transfusion reactions. The re-

TABLE 1. *Examples of Patient Requirements of Leukocyte-Poor Units*

Blood group	Diagnosis	No. of leukocyte-poor units prepared	Period of time (months)
A positive	Hemophilia	33	1
O positive* (anti-rh'' (E)	Lupus erythematosus	11	20
A positive	Congenital telangiectasia	79	12
AB positive	Aplastic anemia	47	35
AB positive	Hypoplastic anemia	18	6
B positive	Aplastic anemia	104	24
A positive	Hypoplastic anemia	68	15
B negative*	Aplastic anemia	48	13
O positive* hr' neg. (anti-K)	Myelofibrosis	52	18

* Deceased

sults obtained with the method[8] used in this laboratory suggest that approximately 57 per cent of nonhemolytic febrile reactions are associated with detectable leukoagglutinins. No attempt has been made here to correlate the demonstration of leukoagglutinins with the number of transfusions each patient had received. However, our results indicate that antibodies against white cells occur more frequently in women than in men, and the observation has been made that they are detected in women in some cases after only one or two transfusions.

It is felt that, where possible, tests for leukoagglutinins should be performed before leukocyte-poor blood is administered. Leukocyte-poor units may be prepared by several different methods, three of which have been tried in this blood bank. The choice of method depends on the volume of such units prepared and will be influenced by individual requirements. Our experience substantiates other reports[2, 4] that the degree of white-cell removal in such units varies with the method used, as well as with unexplained differences in donor blood.[2, 4]

In utilization of buffy-coat-poor units, there is variation in the number of remaining leukocytes that patients may tolerate but the literature[4, 6, 10, 12] and reports received in this laboratory indicate that most patients can take units containing up to 1,500 white cells per cu. mm. of packed red cells.

Acknowledgments

We would like gratefully to acknowledge the assistance and cooperation we have had from Dr. Rose Payne of the Stanford University School of Medicine, and the entire laboratory staff of the Irwin Memorial Blood Bank of the San Francisco Medical Society.

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Book Reviews and Abstracts

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Book Reviews

Surgery of the Aorta and Its Branches. James D. Hardy. Philadelphia, Pa., J. B. Lippincott Co., 1960, 386 pp. $6.50.

THIS MONOGRAPH is a very complete summation of vascular surgery as we know it today. The author has depicted the basic fundamental problems encountered in arterial surgery. His knowledge of diagnosis and management is very complete. The descriptions of operative technic are clearly written and easily understood. The graphic illustrations are extremely explanatory. This is a good text book and should be made available to all surgical residents to assist in teaching the fundamentals of this aspect of surgery.—*Derward Lepley, Jr.*

El Antigeno del Sistema Sanguineo Diego. M. Layrisse and J. Wilbert. Caracas, Venezuela, La Fundacion Creole y la Fundacion Eugenio Mendoza, 1960, 160 pp.

THE MATERIAL covered in this monograph is more extensive than is indicated by its title. The first section of 56 pages is a general discussion of all the blood groups with emphasis on anthropology. The second part (27 pages) is concerned with the genetics and serology of the Diego system. The two final chapters are devoted to a complete presentation of the distribution of the Mongoloid Diego antigen and a discussion of its anthropologic significance. It is not yet possible to explain satisfactorily the apparent absence of Di^a among the Eskimos and the people of Oceania (except for the Sea Dyaks of Borneo), especially the Polynesians.

The format and the organization of the subject matter are good and there appear to be very few errors of which none detracts from the value of the publication. For the sake of complete accuracy the alignment of the Rh loci in Figure 1 on the chromosomes should be in the order DCE rather than CDE and the advisability of depicting F, f and V, v as occupying closely linked fourth and fifth loci may be questioned. Table II would bear some straightening and on page 42 anti-Kp^a and Kp^b should be substituted for anti-Jk^a and anti-Jk^b respectively.

The excellence of the monograph was evident to the reviewer in spite of his handicap in having to read it in broken *English*. It is recommended to anyone interested in the subject who has had moderate success with *Summarios in Interlingua*. We hope that future editions will include generous résumés in English or that the authors are planning an American edition.—*Tibor J. Greenwalt.*

Abstracts

Blood Groups and Antibodies

A Sample of Blood with No Detectable Rh Antigens. G. H. Vos, D. Vos, R. L. Kirk and R. Sanger. King Edward Mem. Hosp. for Women, Perth, Western Australia. Lancet. 1: 14, 1961.

SEVERAL blood samples have been reported whose Rh genotype was described as *D*— —/*D*— —, *DC*^{w}—/*DC*^{w}—, and *Dc*—/*Dc*— respectively. In August 1960, during the routine testing of blood from aborigines living in Western Australia, a sample was found that failed to react with the anti-C, anti-D, anti-E and anti-c sera being used. Fresh samples were obtained for testing with a battery of antisera in two independent laboratories. The blood failed to react with many Rh antisera using saline, albumin, papain, ficin and antiglobulin methods. The cells failed to react with anti-sera of specificities, anti-D, -CD, -C, -Ce, -CE, -C^{w}, -C^{x}, -G, -E, -E^{w}, -cE, -c, -e, -f, -V and -VS. Absorption and elution tests confirmed the negative agglutination tests.

The sample was from a full-blooded Australian aborigine woman in good health. Unfortunately she has no close relatives. According to tribal rules her parents should not be closely related.

This is the first person reported whose blood cells completely fail to react with any known Rh antiserum.—*J. B.R.*

Ge, A Very Common Red-Cell Antigen. R. E. Rosenfield, G. V. Haber, F. Kissmeyer-Nielsen, J. A. Jack, R. Sanger and R. R. Race. Mt. Sinai Hosp., New York City, New York. Brit. J. Haemat. 6: 344, 1960.

THREE examples of a "new" antibody (anti-Ge) which reacted with the red cells of practically everyone tested were obtained from patients in New York City, Houston, Texas, and Aarhus, Denmark. Each example was discovered during routine ante-partum serum testing of pregnant women. The cord blood of all three newborns gave a positive direct antiglobulin reaction of varying intensity. One newborn showed slight jaundice on the third day. No other evidence of overt hemolytic anemia appeared. No treatment was required in any of the newborns.

The specificity of the antibody was studied. The three donors of the antisera and two of their siblings lacked the corresponding antigen, Ge, while 11,500 random Whites, Negroes and Asians were tested and reacted to the antibody, anti-Ge. The antigen, Ge, behaves as a dominant character and is not related to the ABO, MNSs, P, Rh, Kell or Kidd systems.—*A. S.*

A Further Example of Anti-By (Batty) in the Serum of a Woman Whose Red Cells Are of the A_x (A_o) Subgroup of Group A. R. Jakobowicz, J. A. Albrey, W. J. McCulloch and R. T. Simmons. Commonwealth Serum Laboratories, Melbourne, Australia. M. J. Aust. 2: 294, 1960.

A THIRD example of anti-By (Batty) was revealed in routine testing of nearly 1,400 serum samples. This atypical antibody was found in the serum of a woman (Mrs. M) before and following delivery of a By-negative child. The anti-By in Mrs. M's serum was of the incomplete variety demonstrable by the indirect Coombs technic and with papainized erythrocytes. The incomplete anti-By^{a} was present in the cord serum at a titer (1:64) which was identical with that in the maternal serum. The authors classified the anti-By^{a} in Mrs. M's serum as a "naturally occurring" antibody because there was no indication of obvious stimulation; Mrs. M had never received a blood transfusion and Mr. M's erythrocytes were found to lack the By (Batty) factor.

The subgroup of Mrs. M's erythrocytes was classified as A_x (A_o) or C_A. Her cells were (1) agglutinated by all of 13 commercial group O sera, (2) not agglutinated by nine of 17 commercial anti-A sera, and (3) weakly agglutinated by six other anti-A sera. One anti-A serum gave moderate agglutination of her cells while another gave a reaction approaching that given by A_2 cells. Mrs. M's serum contained anti-B (titer = 1-64) and a cold agglutinin with specificity of anti-A_1. Her saliva was found to lack the A substance.—*P. M.*

Application of the Direct Antiglobulin Test to 2,000 Consecutive Specimens of Cord Blood. Evaluation of Positive Tests by Means of Examination of Eluates. P. M. Marcuse and B. J. Francis. Baylor Univ. Coll. of Medicine, Houston, Texas. Amer. J. Clin. Path. 34: 225, 1960.

THE DIRECT antiglobulin test was applied to cord blood specimens from 2,000 successive deliveries. Blood grouping, Rh typing and bilirubin determinations were performed on all specimens demonstrating a positive antiglobulin test. Identification of antibodies was determined by examining eluates prepared from the infants' cells using A_1, B and pooled O cells in the saline agglutinin and indirect Coombs tech-

nics. When an agglutinin against pooled O cells was found, the antibody was further identified by testing the mother's serum against a panel of O cells. Positive reactions were found in 139 of the 2,000 specimens. Only 11 of these positive tests were due to anti-Rh_0 (anti-D) antibody. One hundred and twenty-one positive tests were obtained with bloods in which sensitization of the ABO type was demonstrated.

The application of the direct Coombs test to cord blood samples as a routine procedure gives advance warning of potential hemolytic disease and aids in the early recognition of the mechanism of sensitization. Analysis of eluates prepared from cord blood served to confirm positive direct antiglobulin tests. (This report of the high incidence of positive direct Coombs tests in hemolytic disease of the newborn due to ABO incompatibility offers additional evidence that positive reactions are not nearly so rare as many previous workers have indicated.—Ed.)—*L. R. G.*

A Previously Unrecorded Case of Immune Anti A_1 Antibody in an A_2 Mother. A. Geczy. Welland County Gen. Hosp., Welland, Ontario, Canada. Am. J. Clin. Path. 34: 526, 1960.

THIS is thought to represent the first case of hemolytic disease of the newborn caused by immune anti-A_1 which developed in an A_2 mother.—*H. A. P.*

Rapid Mass Anti-D (Rh_0) Typing with Bromelin. B. Pirofsky, A. August, H. Nelson and R. Pittenger. Univ. of Oregon Med. Sch., Portland, Oregon. J. Lab. Clin. Med. 56: 911, 1960.

A RAPID method for Rh typing was developed. One drop of 0.5 per cent bromelin solution is mixed with two drops of commercial anti-Rh typing serum (diluted 1:6 in saline) in a test tube; an applicator stick of blood is added. The mixture is shaken for 30 seconds, centrifuged and read macroscopically for agglutination.

When 10,000 bloods were tested simultaneously with this and a PVP method, only seven discrepancies, all due to D^u specimens, were noted. A projection of data suggests that the method detects 75-85 per cent of D^u bloods.

Additional experiments showed that concentrations of albumin, dextran and PVP used in blood grouping technics give false positive Rh tests in the presence of bromelin, so that these substances must be well diluted when used with this enzyme. In addition, since bromelin potentiates weak Rh antibodies, commercial anti-Rh_0 sera must be carefully used lest false positives result from contaminating antibodies.—*J. B. R.*

Dangerous Group-O Blood. A Simplified Test. J. W. Lockyer and G. H. Tovey. (South-Western Regional Transfusion Centre, Bristol, England. Lancet. 2: 1369, 1960.

ABOUT 20 per cent of group O donors have demonstrable hemolysins in their sera. These cannot all be considered "dangerous universal donors" and therefore the British Medical Research Council has decided that only those with hemolysin titers exceeding 1:8 be labeled as unsuitable for transfusion of recipients other than O. The authors utilized the observation of Winstanley, *et al.* (1957), that sera containing anti-A hemolysins agglutinate pig red cells of group A^p. A screening test which detects all sera containing hemolysins having a titer higher than 1:8 is described. It correlates well with other procedures including saline titers. By using the porcine A^p test alone, 4 per cent of O donors would be labeled "dangerous."—*T. J. G.*

A New Diagnostic Test for Antiglobulin-Positive ("Auto-Immune") Hemolytic Anemia. B. Pirofsky. Univ. Oregon Med. School, Portland, Oregon. Brit. J. Haemat. 6: 395, 1960.

BROMELIN, an unpurified mixture of proteolytic enzymes obtained from the pineapple stem, was prepared as a buffered 0.5 per cent solution. Two drops of a 2 per cent suspension of patient's cells in patient's serum were mixed with one drop of the bromelin solution. Incubation at room temperature for 15 minutes was followed by slow centrifugation for one minute. Agglutination indicated a positive reaction. Positive reactions were obtained in 42 cases of antiglobulin-positive acquired hemolytic anemia. The direct bromelin test compared favorably with the direct antiglobulin test when 287 control patients with various hematologic disease were studied. No false negative reactions were noted but two false positive reactions due to non-specific cold agglutinins were found.—*A. S.*

Immunology

The specificity of the non-specific. W. C. Boyd. Boston U., Sch. of Med., Boston, Massachusetts. J. Immunology. 85: 221, 1960.

THIS is a good review article which, though brief, points up the more recent findings of sharp blood group specificity of many plant hemagglutinins previously assumed to be non-

specific. The meaning and relationship of specific inhibitory characteristics of certain carbohydrates to prevent the reaction of plant agglutinins with all or some human erythrocytes is discussed. This carbohydrate inhibitory function is related to probable chemical structure of the receptors in the human red cell. Studies led to the classification of aldopyranoses into four groups based upon their configuration at carbon atoms three and four. These studies tend to emphasize the role of galactose as a terminal unit in some of the human red cell receptors. The inhibition reactions have also been applied to anti-Rh sera and similar conclusions are suggested. The relationship of this type of experiment to the elucidation of antigen chemical structure is discussed.—*A. S.*

The production of antibody by tranfused bone marrow. I. L. Stoloff. Jefferson Med. Coll., Philadelphia, Penna. J. Immunol. 85: 304, 1960.

BONE marrow cells and spleen cells from mice hyperimmunized with tetanus toxoid were capable of continuing the production of tetanus antitoxins after transfer to irradiated hosts. Isologous cells functioned well 25 days after transfer, but there was a sharp decline in the production of antibody by homologous cells after the first week of transplantation. The use of this technic as a measure of cell survival in marrow transplants and its limitations is discussed.—*A. S.*

Identification of blood group antigens and minor cell populations by the fluorescent antibody method. F. Cohen, W. W. Zeulzer and M. M. Evans. (Children's Hosp. of Michigan, Detroit, Michigan.) Blood. 15: 884, 1960.

THE fluorescent antibody technic was applied to (1) the identification of red blood cell antigens, and (2) the investigation of minor erythrocyte populations *in vitro* and *in vivo*. Fluorescent antibody reagents were prepared by conjugating ammonium sulfate precipitated antisera with fluorescein isothiocyanate. The test systems consisting of red cells and labeled antibody were examined microscopically as wet preparations; dark field and ultraviolet illumination were employed alternately.

When A_1 and A_2 erythrocytes were treated separately with fluorescent anti-A, clumping resulted and a vivid light green fluorescence was observable under ultraviolet illumination. Smaller clumps and more single cells were formed with A_2 cells than with A_1, and the intensity of specific fluorescence of A_2 cells was much weaker and less uniform than that of A_1 cells. Group B cells treated with labeled anti-B gave similar results as that of the A_1-anti-A system. In control experiments, group O cells and B cells failed to show fluorescence when treated with labeled anti-A serum; group O and A cells gave negative results with labeled anti-B serum. Group A_1 or B cells did not fluoresce when treated with an AB serum conjugated with fluorescein isothiocyanate.

Experiments were included to show that the agglutinates contained some non-specific protein. However, this nonspecific trapping of fluorescent protein was distinguished from the appearance produced by antibody protein specifically attached to red cells and was not a factor in intensifying specific fluorescence.

Experiments in which labeled anti-Rh_o was incubated with Rh positive cells failed to result in the production of fluorescence; however O Rh positive cells sensitized with incomplete anti-Rh_o and treated with labeled anti-human globulin rabbit serum fluoresced to a degree similar in intensity to that of the A_2-anti-A system. Likewise the hr′, rh″, and Jk^a factors were specifically demonstrated by employing the appropriate unlabeled antiserum and fluorescent anti-human globulin reagent; the intensity of fluorescence was equal to or less than that obtained with the Rh_o-anti-Rh_o system.

In applying the fluorescent antibody technic to the detection of minor cell populations in artificial mixtures of erythrocytes, the authors were able successfully to differentiate A cells (minor) from O cells, B cells (minor) from O cells, A cells (minor) from B cells, B cells (minor) from A cells, Rh_o positive cells (minor) from Rh_o negative cells and hr′ positive cells (minor) from hr′ negative cells. Minor cell populations were demonstrated in red cell mixtures with ratios (minor:major) ranging from 1:10 to 1:100,000.

The fluorescent antibody technic was employed to detect minor cell populations in the blood of a B, Rh negative donor. Following injection of the volunteer with O Rh_o positive cells, fluorescent erythrocytes were demonstrable in the recipient's blood specimens for as long as 20 days. Six days after he received the O Rh positive cells, the volunteer was injected with A_1 Rh negative cells. Employing fluorescent anti-A serum, the authors found that only a few fluorescent group A cells could be seen in specimens obtained at six minutes and only a very rare fluorescent cell was observable in a 24-hour specimen. The authors also reported on

their initial experiments in which they applied the fluorescent antibody technic to study the transplacental passage of fetal erythrocytes. This experimental tool should prove invaluable in elucidating the mechanism of hemolytic disease of the newborn.—*P. M.*

A sensitive quantitative hemolysin test. Its application in the study of anti-A and anti-B antibodies. R. N. Ray and H. Chaplin, Jr. Division of Hematology, Departments of Medicine and Preventive Medicine, Washington University School of Medicine and the Barnes Hospital, St. Louis, Missouri. Am. J. Clin. Path. 34: 277, 1960.

A QUANTITATIVE hemolysin test has been designed in which dilute suspensions of test red blood cells are employed. The concentration of red cells in the test suspension may be decreased by 100 fold from conventional suspensions without loss of accuracy. The AB serum which served as a complement source could be stored as long as four months at −20 C. without appreciable loss of activity in dilutions of 1:8 or less. Measurement of hemoglobin concentration was performed by a modified benzidine method designed to measure hemoglobin in plasma. The maximal hemolytic action of anti-A serum occurred within 15 minutes. A_1 cells were more susceptible to hemolysin than A_2 cells. A_1 cells from a patient with paroxysmal nocturnal hemoglolimuria were more readily hemolyzed than normal cells. This susceptibility of red cells to hemolysins was not altered by storage in ACD up to two weeks. No hemolytic activity was observable when potent, incomplete Anti-D serum was tested with both trypsinized and normal D positive red blood cells.—*L. R. G.*

Specificity and Inheritance of Antibody Response: A Possible Steering Mechanism. B. Cinader (Department of Medical Biophysics, University of Toronto). Nature. 188: 619, 1960.

INJECTION of a heterologous protein into a newborn animal prevents antibody formation to it for part or all of the animal's life. This acquired immunologic tolerance has been induced in rabbits, calves, goats, mice and birds with serum albumins, globulins, ribonuclease, azo serum albumin, and yeast antigen. It is suggested that "tolerance" is as universal as "response." Failures to induce tolerance have been frequent when red cells are used. The dose used to produce tolerance must be sufficient to supply enough antigen to assure contact with every antibody producing cell or its mother cell. If only a relatively small proportion of these cells has a tolerance-inducing encounter with antigen, the slight decrease in antibody production may be masked. On the other hand if only a small proportion of the antibody producing cells are unaffected, prolonged immunization would be required before these have multiplied sufficiently to produce measurable levels of antibodies.

The presence of impurities in the antigen may lead to misleading results because it may appear that tolerance was not produced to the major antigen whereas the result may be due to the failure of the impurities to achieve this goal. This difficulty may occur when heterologous cells not capable of multiplication are injected at birth.

Tolerance to one heterologous antigen can influence antigen-antibody response to another one, if an immunologic determinant is shared by both. In case of autologous proteins an adult animal would not only be prevented from making antibody to the autologous protein as such but also to any determinant of the autologous protein. It is thus not necessary to assume that response to all antigens is under direct genetic control but may be mediated through the consequence of acquired tolerance to autologous proteins. The variability of response in different individuals may depend then on genetic protein differences.—*T. J. G.*

Studies of Blood Group Antibodies. I. Fractionation of Anti-A and Anti-B Isohemagglutinins by Anion-Cation Cellulose Exchange Chromatography. III. Observations on the Physicochemical Properties of Isohemagglutinins and Isohemolysins. IV. Physicochemical Differences between Isoanti-A, B and Isoanti-A or Isoanti-B. A. J. Rawson and N. M. Abelson. School of Med., Univ. Pennsylvania, Philadelphia, Pennsylvania. J. Immunol. 82: 435, 1959 and 85: 636, 640, 1960.

BY THE USE of modified cellulose anion and carboxymethyl cellulose cation exchange adsorbents, anti-A and anti-B serum specimens were fractionated. The sera studied included some from normal subjects who had no history of antigenic stimuli, from subjects who had previously been stimulated by A and B blood group specific substances or an incompatible blood transfusion, and from mothers of infants with presumed major group hemolytic disease.

Iso-hemagglutinins, anti-A and anti-B, were separated into three components. One fraction of low anionic binding capacity is associated with gamma-2 globulins with a sedimentation constant of the 7S class. This fraction has serologic activity of the "immune" type. A second fraction of intermediate anionic binding capacity consists of an albumin-globulin mixture. The globulin has the mobility of alpha-2 globulin and the sedimentation constant of the nonalbumin portion is about 15.2S. The third, a high anionic binding fraction, contains some non-gamma globulin plus a serologically-active gamma-1 globulin with hemolysin activity. This gamma-1 globulin fraction is not homogenous with respect to sedimentation constant and contains 3.5S, 7S and 19S globulins.

Isohemolysins were found associated with 7S gamma-2 globulins and 19S gamma-1 globulins.

When the sera of individuals of group O were compared with those of group A and group B a difference in physicochemical type was noted. Both before and after specific antigenic stimuli, blood group O individuals tend to produce 7S gamma-2 globulin type antibody while those of blood groups A and B tend to produce 19S gamma-1 globulin type isoagglutinins. Since 7S globulin more easily traverses the placental barrier than does 19S, these differences in antibody pattern may provide a partial explanation for the predominance of group O women among mothers of infants with ABO hemolytic disease.—*A. S.*

Immunologic Differences between the 7S and 19S Components of Normal Human Gamma Globulin; Antigenic Specificity of the 7S Component. E. C. Franklin. N. Y. U. College of Medicine, New York City, New York. J. Immunology. 85: 138, 1960.

7S GAMMA globulin free of heavy components was prepared from Cohn fraction II gamma globulin by ultracentrifugation. Heavy components (19S and greater) were similarly prepared and both were independently purified by zone electrophoresis in starch. Double diffusion studies in agar and quantitative precipitin studies confirmed the close antigenic relationship of 7S and 19S fractions. Moreover antigen differences of groups specific to the 7S gamma globulin were detected. Heavier components in these preparations and in normal serum do not appear to be simple polymers of 7S gamma globulin.—*A. S.*

Transmission of Serum Proteins from Foetus to Mother in the Rhesus Monkey. D. R. Bangham, K. R. Hobbs and D. E. H. Tee (National Institute for Medical Research, Mill Hill, London, N.W. 7). Lancet. 2: 1173, 1960.

^{131}I-LABELED homologous fetal serum proteins were injected intravenously and intraperitoneally into the fetus during mid-pregnancy. Labeled albumin and gamma globulin isolated by electrophoresis and chromatography were found in the maternal serum two to eight days afterwards. There is apparently a constant exchange of these proteins between the fetus and the mother which presumably takes place across the placenta because only negligible amounts of radioactive protein were found in the amniotic fluid. Previous studies also showed that little of the protein found in the liquor amnii in mid-pregnancy can come from the fetus. In the Rhesus monkey the placental system is closely similar to that of the human.—*T. J. G.*

Auto-Antibodies and Lupus Erythematosus. (Editorial.) Brit. Med. J. 2: 1141, 1960.

"ALTHOUGH the study of the lupus cell phenomenon has proved disappointing in achieving a more precise definition of the disease, it has been a stimulant to the elucidation of the disordered immunological process. The lack of correlation between the quantity (titers) of the anti-nuclear and anti-cytoplasmic serologic factors on the one hand and the extent of the disease on the other has led most authors to believe that these reactions are a manifestation of an unusual state of the antibody producing system rather than a cause of the disease." This excellent article reviews in three concise pages the historical evolution of our current concepts of lupus erythematosus, firmly delineates the variety of immunologic abnormalities, and critically examines the role of these supposedly "auto-antibodies" in the light of recent clinical evidence. "Currently, in one series of 100 patients over half are alive at least ten years after the onset of the disease, and of these many are not at all incapacitated, some in complete remission." The fact that recoveries do occur is one of the reasons for supposing that the "auto-antibodies" in lupus, in the opinion of the editors, are a result rather than the cause of the disease.—*H. F.*

The Plasma Proteins in Normal Urine. I. Berggard. Univ. of Upsala, Upsala, Sweden. Nature. 187: 776, 1960.

USING specific antisera against purified proteins in immuno-electrophoresis and in Ouchterlony double diffusion methods, the author has

convincingly demonstrated the presence in normal urine of proteins immunologically identical with the plasma proteins: pre-albumin, albumin, ceruloplasmin, transferrin and gamma globulin. —*H. F.*

Storage and Transfusion

Plasma Volume Expansion and Reactions after Infusion of Autologous and Nonautologous Plasma in Man. J. L. Hutchison, S. O. Freedman, B. A. Richards and S. V. Burgen. Montreal Gen. Hosp., Montreal, Quebec, Canada. J. Lab. Clin. Med. 56: 734, 1960.

THE AUTHORS present evidence that reactions to nonautologous plasma, apparently allergic in nature, occur with surprising frequency and are associated with a failure of the circulating plasma volume to rise to the degree anticipated by the amount of plasma infused. Five hundred ml. of citrated plasma were infused into normal recipients after a few days' storage at −23 C. Six of the 15 recipients of nonautologous plasma developed urticaria. Four of these had giant hives accompanied by systemic symptoms (chills, headache, nasal stuffiness, faintness, or joint pains). The mean rise of plasma volume in this group was only 18 per cent of that expected; the most severe reactions were associated with a decrease.

In contrast, when autologous plasma was infused the mean rise in plasma volume was 83 per cent of that anticipated. Dextran resulted in an 82 per cent increase.

When the recipients were skin-tested with plasma, eight of ten had a larger wheal with nonautologous plasma than with their own; however, there was no correlation between the results of the skin tests and the response to plasma infusions.—*H. A. P.*

Platelet Transfusions and Administration of Platelet Derivatives in Man. I. Evaluation of Laboratory Technics Used for Prognostic Purposes. II. Evaluation of Systemic Hemostatic Properties of Various Preparations. G. Raccuglia and F. H. Bethell. Univ. of Michigan Med. Sch., Ann Arbor, Michigan. Am. J. Clin. Path. 34: 495, 505, 1960.

SEVERAL laboratory tests, *viz*, thrombelastography, clot retraction, serum prothrombin time, platelet counts, capillary fragility test and bleeding time, were used to estimate the severity of bleeding in thrombocytopenia. Only the bleeding time (Ivy method, normal value of less than seven minutes) was found to parallel the patient's clinical condition. Patients with a bleeding time longer than 30 minutes were believed to be in imminent danger of acute hemorrhage and therefore in need of systemic therapy. The bleeding time was used serially to determine the results of therapy.

Systemic therapy consisted of the intravenous administration of platelets using plastic collection, separation and administration equipment. In various experiments platelets were administered as concentrates (Klein, *et al.*) either fresh (within 24 hours of separation) after ordinary refrigeration or freezing, or after freeze-drying, preservation in gelatin, ultrasound disintegration or degradation into a phospholipidic preparation.

Optimal hemostatic effects were obtained only when whole platelets less than 24 hours old were administered and required the platelets derived from four blood donations. With the possible exception of gelatin preservation, the other methods were uniformly ineffective. Because of the necessity of preparation of fresh material, a "platelet bank," *i.e.*, a reservoir of platelets available at any time for immediate use, is still not practicable. Nevertheless, since platelets are the only material of proven usefulness in the management of acute hemorrhage in non-immune thrombocytopenia, a modern blood bank should be prepared to furnish platelet concentrates.—*J. B. R.*

The Use of Human Albumin During Exchange Transfusion. W. H. Kitchen, V. I. Krieger and M. A. Smith, Royal Women's Hosp., Melbourne, Australia. Med. J. Australia. 2: 781, 1960.

EXPERIMENTS reported by others have shown that human albumin has an avid binding capacity for indirect-reacting bilirubin; in animals the incidence of kernicterus is reduced by the administration of albumin; and bilirubin diffuses readily into the circulation from extravascular spaces during exchange transfusion.

In a small but carefully controlled group of erythroblastotic newborns, salt-poor human albumin was used as an adjunct to exchange transfusion. Fifty ml. were administered at the beginning of the transfusion and again when the exchange was half completed. There was a statistically greater amount of bilirubin removed and fewer re-exchanges were necessary in the albumin-treated infants as contrasted to infants who did not receive added albumin.—*J. B. R.*

A Blood-Transfusion Bottle Heater. G. Griffith (St. David's Hospital, Bangor). Lancet. 2: 1174, 1960.

THE BOTTLE heater developed by Electrothermal Engineering, Ltd., 270, Neville Road, London, E. 7, has been used to bring and maintain a bottle of blood at 37 C. during exchange transfusions. It is essentially a snug, insulated, thermostatically controlled jacket of 50 watt capacity operating on a 230/250 volt current and has been found satisfactory in two years' use.—*T. J. G.*

Stability and Quality of Plastic Transfusion Equipment. C. W. Walter, L. N. Button, C. Greggory and J. G. Gibson, II. Peter Bent Brigham Hosp., Boston, Massachusetts. Surg., Gyn., Obst. 111: 170, 1960.

STUDIES are reported on donor blood plastic bags which had been stored for as long as four years prior to use. Results showed excellent uniformity of the product over this period. The volume and content of the contained ACD solution remained well within the limits of NIH requirements. Trace elements in both plastic and solution remained unchanged during the period of storage, and there was no evidence of leaching of undesirable materials from the container wall into the ACD.

Post-transfusion survival of red blood cells after 21 days of storage remained the same regardless of the age of the bag. The mean survival was 83 per cent. Although two samples showed less than 70 per cent post-transfusion survival, the variation between results of individual experiments appears compatible with those found in other biological experiments. Overall results were definitely superior to survivals obtained with blood stored in glass bottles reported from other sources.—*H. A. P.*

Hemorrhage—Anticipation and Control. Preoperative Detection of Bleeders, Local Hemostasis During Operation, and Problems of Transfusion. J. A. Buckwalter and W. E. Connor (College of Medicine, State University of Iowa, Iowa City). J.A.M.A. 174: 151, 1960.

ESSENTIALLY all congenital coagulation defects and most acquired defects can be detected by taking a proper history and doing a careful physical examination before operation. The practice of determining clotting and bleeding times as part of the pre-operative routine may cause harm because of mistaken confidence in the tests. When the patient is a "suspect" bleeder, the authors recommend a detailed series of coagulation studies including Lee-White coagulation time, Ivy bleeding time, capillary fragility, clot retraction, fibrinogen value, fibrinolysis, one-stage and two-stage prothrombin times, prothrombin consumption; platelet count; circulating anticoagulants and thromboplastin generation tests. The use of stored blood, fresh whole blood, fresh plasma, platelet and fibrinogen transfusions is briefly discussed. Mention is made of the new sulfhydryl-substituted methylnaphthoquinone, vitamin K-S (II) which is supposedly of value in the correction of Factor V and VII deficiencies in cirrhotic patients. The use of other "natural" and synthetic vitamin K preparations is described. The authors feel that oxycel has no advantages over absorbable gelatin sponge and prefer bovine thrombin over human thrombin and thromboplastin preparations in local hemostasis. They have not been impressed with the effectiveness of carbazochrome preparations. The authors most appropriately stress the importance of the control of anatomic bleeding and deplore the use of single unit transfusions.—*T. J. G.*

An Evaluation of Blood-Transfusion Practices on a Surgical Service. J. H. Morton (University of Rochester School of Medicine and Dentistry, Rochester, New York). New Eng. J. Med. 263: 1285, 1960.

DURING the period covered 169 or 36 per cent of 475 adults transfused on the surgical service received a single unit. Ten per cent of the one unit transfusions were classed as "conservative" because actually administration of two or three pints might have been justified; 18 per cent were "reasonable" because massive blood loss was expected but did not occur; 38 per cent were "questionable" since the transfusion might have been withheld although there was some suggestion of hypovolemia; 34 per cent were classed as "unnnecessary" because no valid indication could be elicited. A brief review of the problem and its importance is given. It is most welcome to note that an increasing number of surgeons are addressing themselves to this problem.—*T. J. G.*

Radiochromium in the Estimation of Survival of Red Blood Cells. Review of the Literature. A. C. Aufderheide. St. Mary's Hosp., Duluth, Minnesota. Amer. J. Clin. Path. 34: 258, 1960.

THE USE of radiochromium in the estimation of the survival of red blood cells is presented as a practical clinical and investigative procedure

when employed within its limitations. Factors affecting the technics of labeling and counting as well as the clinical applications are reviewed. The bibliography is quite complete and should be of a great value to workers interested in pursuing the subject.—*L. R. G.*

Blood Donors and Recipients

Incidence of Serum Hepatitis in Recipients of Thymol Turbidity-Screened Blood or Aged Liquid Plasma. N. G. Maxwell. Milwaukee Blood Center, Milwaukee, Wisconsin. Am. J. Clin. Path. 34: 518, 1960.

CAREFULLY standardized thymol turbidity tests were performed on a large series of donor bloods. Recipients, actively traced and checked for the occurrence of hepatitis, had an incidence of 0.685 per cent when the donor blood had an elevated thymol turbidity and 0.692 per cent with normal values for the test. Twelve donors whose blood was transfused into recipients who subsequently developed hepatitis (four resulting in two cases each) had no demonstrable abnormality by a variety of liver function tests. Pooled plasma held at *room temperature* for six months resulted in the same incidence of hepatitis as whole blood.—*H. A. P.*

The Prevalence of an Increased Isocitric Dehydrogenase (ICD) Activity in a Segment of the Blood Donor Population with a Low Social and Economic Status. G. N. Bowers, Jr., H. P. Potter, Jr. and R. F. Norris. Medical School, Univ. Pennsylvania, Philadelphia 4, Pennsylvania. Am. J. Clin. Path. 34: 513, 1960.

THE LEVEL of isocitric dehydrogenase (ICD) in the serum is believed to be elevated above the normal range only in the presence of liver disease. The results of tests on the serums of blood donors who were prison inmates or "walk-in" professional donors were compared with those of a control group of volunteer donors (including relatives of patients).

The incidence of increased ICD, increased bromsulfalein retention, diminished serum albumin, and chronic alcoholism was greater by a highly significant degree in the prison inmate and "walk-in" groups. The results suggest a greater incidence of chronic hepatic disease in the lower socio-economic group. No direct evidence is presented that this group has a higher incidence of carriers of viral hepatitis. Correlation of ICD levels in donors with the occurrence of hepatitis in recipients will be necessary to establish the value of ICD levels as a screening test for hepatitis carriers among donors.—*H. A. P.*

Leukocytes

A study of some factors influencing leukocyte agglutination *in vitro*. A. K. Saraya and S. Kumar. K. G. Med. College, Lucknow Univ., Lucknow, India. Ind. J. Med. Res. 48: 577, 1960.

LEUKOCYTE agglutinins in sera from immunized rabbits were studied. Optimal results were obtained with an antigen concentration of 20,000 per cmm., at a temperature of 27 C. and when centrifugation was employed. Leukolysis could not be produced by adding guinea pig complement to the test mixtures.—*J. B. R.*

Leukokinetic studies. II. A method for labeling granulocytes *in vitro* with radioactive diisopropylfluorophosphate (DFP^{32}). A. M. Mauer, J. W. Athens, H. Ashenbrucker, G. E. Cartwright and M. M. Wintrobe. Univ. Utah Coll. of Med., Salt Lake City, Utah. J. Clin. Invest. 39: 1481, 1960.

THE AUTHORS describe a technic of labeling granulocytes in 500 ml. of whole blood which permits following their post-transfusion survival. Fifty-four per cent of the labeled cells disappeared from the circulating blood during the period of infusion; the remainder were lost in random fashion with a mean half life of 6.6 hours. The proportion of infused cells remaining in the circulation decreased to 12 per cent if the blood was stored for 24 hours.

Transfusion of labeled blood to ten normal recipients other than the donors resulted in accelerated loss of granulocytes in two instances, even though the recipients had not been previously transfused.—*H. A. P.*

Familial neonatal neutropenia with maternal leukocyte antibodies. E. H. Braun, A. E. Buckwold, H. E. Emson and A. V. Russell. Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada. Blood. 16: 1745, 1960.

AFTER her second pregnancy a woman was given a transfusion of fresh whole blood donated by her husband. Three out of the subsequent five children had evidence of neutropenia.

During the seventh pregnancy, leukocyte agglutinins against the cells of her husband, a previously affected child and three out of six unselected donors were demonstrated and rose

in titer. Leukolysins appeared four days prior to delivery. Leukoagglutinins appeared in the cord serum. The maternal leukoagglutinins could be eluted from positive leukocytes.

The seventh child exhibited an initial neutropenia which became more marked between the twentieth and fortieth days of life. On the eighth day, bone marrow aspiration showed an almost complete lack of white cell precursors. Moniliasis developed within the first week but responded to vigorous treatment. No other cellular elements of the baby's blood (outside of the neutrophils) were diminished.—*J. B. R.*

Miscellaneous

Nickel sensitivity as a cause of infusion reactions. J. C. Stoddart. R. A. F. Hospital, Ely, Cambs, England. Lancet. 2: 741, 1960.

SKIN reactions developed in two patients who had had infusions of plasma, blood and intravenous solutions and in one of these there was also an alarming anaphylactoid reaction. It is suggested that the cause was sensitivity to nickel in the infusion cannulae. As nickel sensitivity is relatively common, a proportion of the hitherto unexplained infusion reactions may be due to nickel cannulae, which may produce manifestations of the immediate or delayed type of hypersensitivity.

Three cannulae and one metal gauze filter (in a recipient set) were analyzed. All were found to be made of substantial amounts of nickel.—*J. B. R.*

Absorption-elution grouping of dried blood stains on fabrics. S. S. Kind. Home Office Forensic Science Lab., 32 Rutland Dr., Harrogate, England. Nature. 187: 789, 1960.

A METHOD is presented for identifying ABO groups from dried blood on pieces of cloth using an absorption-elution technic.—*J. B. R.*

Venous thrombosis during dextrose infusion. D. W. Vere, C. H. Sykes and P. Armitage. The London Hospital, London, England. Lancet. 2: 627, 1960.

FIFTY-NINE patients were given intravenous infusions of dextrose solutions. Twenty-nine (or about 50 per cent) of these patients developed thrombophlebitis. The incidence was higher with autoclaved than with filtered solutions; this was believed due to an increase in acidity of the autoclaved solutions. When dextrose-saline was given after a blood transfusion through the same recipient set, the incidence of thrombophlebitis increased. It was noted that the cells remaining in the recipient set filter were hemolyzed.

In vitro studies showed that fresh red cells are readily lysed by glucose solutions after contact at room temperature for four hours.—*J. B. R.*

The "toxin-antitoxin" phenomenon in burned and injured human subjects. S. R. Rosenthal, J. B. Hartney and W. A. Spurrier. Cook County Hosp., Chicago, Ill. J.A.M.A. 174: 957, 1960.

IN 1958, in a catastrophic Chicago school fire a number of children were severely burned. Nine of these children were given whole blood and plasma collected from donors who themselves had previously suffered severe burns. The clinical results were very gratifying. Clear-cut results were seen when at least 500 ml. of whole blood or 250 ml. of plasma were given in a 24-hour period. In some a "crisis" seemed to occur within hours after the transfusion. Improvement included diminution of irritability, gain in appetite, lessened pain, drop in temperature, decrease in urinary albumin output and ability to move extremities, etc.

Evidence for the presence of a "toxin" in the blood of burned patients includes demonstrations of a cytotoxic effect on HeLa cells, a cytolytic effect on red cells and precipitogens. Similarly, the sera of convalescent burned patients can be shown to contain anticytotoxins, hemolysins and precipitins for five years after burning. These are believed to be true antibodies.—*J. B. R.*

Status of human plasma as a plasma volume expander. H. F. Hamit. Letterman General Hosp., San Francisco, California. J.A.M.A. 174: 1617, 1960.

ENTHUSIASM for the military use of plasma as a blood substitute began in 1939. To make its use more practical pooling and freeze-drying were adopted but the latter measures encouraged the development of serum hepatitis. To combat hepatitis, ultraviolet irradiation was advocated and then discredited. Other measures such as chemical sterilization and prolonged heating have been used but prolonged shelf storage has proved the most satisfactory method of rendering plasma non-icterogenic.

Human plasma is still the best plasma expander. Some of the synthetic polypeptides show promise and animal plasma products are being used abroad. The most satisfactory products

now available for stockpiling are serum albumin, a commercial plasma product and liquid plasma. No mechanism yet exists for the stockpiling of liquid plasma on a national basis. The demand for its use almost outstrips the supply.

The cause of homologous serum hepatitis is shrouded in mystery. A simple method for detecting the carrier of this disease or of sterilizing whole blood is needed. In the meantime efforts to reduce the incidence of this disease in transfusion practice will be largely based on empiricism.—*J. B. R.*

A Proposed Standard System of Nomenclature of Human Mitotic Chromosomes. A. Robinson (University of Colorado Medical School, Denver 20, Colorado). J.A.M.A. 174: 159, 1960.

THE RATHER phenomenal recent growth of knowledge of human chromosomes and the increasing number of publications concerning them has already led to confusion because of the lack of a common system of nomenclature. A meeting of a small group of experts was called in order to formulate a common system of nomenclature. This concise summary of the results of this meeting will serve as a useful guide to all those interested in reading and understanding the articles published on this subject.

It is recommended that the term, karyotype, should be applied to a systematized array of the chromosomes of a single cell prepared either by drawing or by photography. The term, idiogram, would then be reserved for the diagrammatic representation of a karyotype, which may be based on measurements of the chromosomes in several or many cells. It was agreed that the autosomes should be serially numbered 1 to 22 as nearly as possible in descending order of length. The sex chromosomes will be referred to as X and Y rather than by a number. It was generally agreed that the 22 autosomes can be classified into seven groups. A table describing the seven groups of chromosomes is presented. Additional tables furnish the quantitative characteristics and the synonyms which authors have already published.—*T. J. G.*

Trend of Charity Immunity Rule. P. R. Overton. 1905 North Larmar, Austin, Texas. Texas Hospitals 16: 32, 1961.

THE SUPREME COURT of Michigan, September 1960, in the case of *Parker vs. Port Huron Hospital,* held that the charitable immunity rule would no longer make it possible for a hospital to avoid liability for negligent acts of its employees. The Supreme Court affirmed the decision by awarding $20,548 to the plaintiff. The case involved the improper identification of a pretransfusion blood sample which resulted in the administration of Group A blood to a Group O patient. The patient died 13 days later.—*L. R. G.*

Questions and Answers

Fresh *Versus* Old Blood

Q. "As a member of the Pima County Medical Society and Medical Advisory Committee to the Southern Arizona Regional Council of the American Red Cross, I have been asked to inquire of your group the present status in regards to 'aging' of blood. Specifically, our group was presented the problem recently of the various classifications in blood usage within the normal accepted 21 days in which blood is generally kept and made available for transfusion. For example, it is our policy to supply blood for exchange transfusion for pediatric patients that is no older than four days because of the known increase in potassium ion concentration after that time. We all seem to be in agreement about this point. However, recently, the surgical group has advised us that they would like 'fresh blood' because of their feeling that this blood was better able to help and control hemostasis by controlling oozing of blood from the surgical incision. We are aware that there is a destruction of platelets in transfused blood but since this area does not supply blood in plastic bags or in silicone coated bottles unless it is by special arrangements, we felt that the problem of hemostasis in relation to blood transfusion did not exist until it was brought to our attention by the surgical group. We are quite anxious in knowing of your position on this matter and of knowing any reference to investigated work that might substantiate or refute their claim that 'fresh' blood was a better hemostatic agent.

Finally, we have been told by the medical group that there is a greater incidence of 'sludging' in the blood that is well on toward the twenty-first day of age. This blood is more difficult to force through the blood filter and is apt to be discontinued and returned to the

laboratory without the full volume of blood being administered because of the sludging effect. The medical group also feels that there may be a higher incidence of transfusion reactions in the blood that is nearing its expiration date."—*M.D., Tucson, Arizona.*

A. "1. Blood not over five days old should be used for exchange transfusion in erythroblastotic infants.

2. Fresh blood falls into various categories depending upon the defect in the patient which needs correction. Blood of any acceptable period of storage may be used for the relief of acute blood loss. Blood not over four hours old should be used for the correction of platelet deficiencies or for the preparation of plasma for the treatment of hemophilia. There is evidence, particularly from Krevans of Hopkins, that blood collected in ordinary blood bottles is satisfactory for the preservation of platelets. However, gravity collection in a plastic bag from a free-flowing phlebotomy assures maximal concentration of platelets and other coagulation factors. It is certainly true that blood can be collected in ordinary glass containers for its anti-hemophilic factor. In hypoprothrombinemia or other undetermined or ill defined coagulation difficulties, blood less than 24 hours old will have many labile factors which are not present in older blood.

3. In patients undergoing rapid, massive transfusions, non-fresh bank blood can dilute the patient's platelets and cause secondary thrombocytopenia. It is good practice to have a reasonable percentage of fresh blood, that is blood which is less than 24 hours old, probably to the extent of between 10 and 20 per cent of the blood given when the transfusions in a 24 hour period may amount to an exchange transfusion.

4. The use of fresh blood, and in this case one would mean blood not over several days old, is recommended when the recipient must depend on transfusions for survival in the course of severe anemia due to bone marrow hypoplasia.

With regard to the so-called 'sludging' of blood that is nearing its expiration date, this is not a problem if an adequate filter is used and changed frequently enough. A very large experience with transfusions of packed cells has shown the ease with which even such blood with an average hematocrit of 70 per cent can be given into any vein that will admit an 18 or a 19 gauge needle.

There is no documented evidence of a higher incidence of transfusion reactions in blood that is nearing its expiration date. Some blood banks have used considerable quantities of blood up to a week beyond the accepted 21 day expiration date, particularly for acute blood loss, without harm to the recipient. There is nothing magical which occurs at midnight on the twenty-first day after blood has been drawn which converts 'good' blood into 'bad' blood." —*Mark Falcon Lesses, M.D.*

Fractional Units and Pilot Samples

Q. "Recently questions have been raised regarding our policy of using fractional units, and I feel that official clarification is desirable. If a fractional unit is ordered and given, the remainder of that unit is considered to have an expiration time of three hours, similar to that of packed red cells on page 13 of *Standards.* The question is whether this policy is too strict and should be modified or whether it is correct.

The problem is further compounded by certain local situations. In our hospital, inpatients are not charged for laboratory work. As a result the physicians on our staff are accustomed to ordering blood for crossmatch to meet their potential needs rather than to ordering a crossmatch only when blood is to be transfused. As a result, orders for crossmatches outnumber the units of blood given by a factor of 3. As a result some bloods may be crossmatched ten or more times before a unit is given. Pilot samples which we draw are adequate to handle this load, but blood obtained from other sources frequently have pilot samples inadequate for this number of crossmatches. There are, therefore, occasions when a 'bottle sample' must be taken to use for crossmatch purposes."—*M.D., Cincinnati, Ohio.*

A. "You have raised the question as to whether the three hour expiration time is too strict after a blood container has been entered for the purpose of withdrawing a fractional unit or obtaining blood for a crossmatch.

The Standards Committee of the American Association of Blood Banks has had this matter under active consideration and has modified the 1958 'standards.' (See 1960 Edition.)

If bottles such as are in current use are entered under conditions of strict asepsis, if a bacteria-excluding air vent is utilized, and if proper refrigeration is continuously maintained, then it is felt that 24 hours is a safe expiration period. If lacking any one or all of these provisos, then three hours should still be considered the safe expiration period.

On the other hand transfusion equipment is now available which will allow safe aseptic withdrawal of blood from a container and permit a 21 day expiration period of the residual blood or cells. This equipment is more expensive than bottles, but is more flexible.

Your second problem dealt with the situation caused by inadequate pilot samples on blood received from other sources. There is no doubt that in most blood banks many more crossmatches are performed than bloods are transfused. This may at times be overdone, but in general, especially for the operating room, it is wise to crossmatch in advance and in excess of use.

The entrance of a bottle for the securing of a pilot sample is just as hazardous as its entry for any other purpose and of course shortens the expiration time of the blood to three hours under the current standards and to 24 hours under the projected standards. Obviously there will always be enough red cells in an attached pilot tube for a very large number of 'major' crossmatches, even ten or more. I may say parenthetically after reviewing our own experiences involving some 3,000 transfusions yearly, that it is quite rare to crossmatch a given unit of blood more than six times and that the vast majority do not go through more than four crossmatches: this is associated with a maximal 48 hour reservation period and a 5 per cent outdating.

Your problem with the inadequate or used up pilot sample, therefore, comes because you cannot perform a 'minor' crossmatch utilizing the donor's serum against the recipient's cells. One very simple method that avoids entering the bottle for a fresh pilot sample is to omit the 'minor' crossmatch when the amount of serum in the pilot sample is low. This of course is easy to estimate if you routinely separate cells from serum in the pilot tube. Instead of a 'minor' crossmatch in these cases the final supply of serum is retested against A cells and B cells and crossmatching by indirect Coomb's or in a high protein medium or by Low's papain technic against a panel of group O R_1R_2 cells containing the most common blood factors such as Kell, Duffy, Kidd, etc. The 'minor' crossmatch in any event is chiefly important to reconfirm the ABO groups and to identify the rare atypical antibodies occurring in donor blood. Here again, if a plastic bag is used with integral donor tubing an adequate pilot sample can always be secured without risk of contamination of the blood in the primary container."—*Mark Falcon Lesses, M.D.*

Donor Antibodies

Q. "Is the routine screening of blood donors for the presence of irregular antibodies indicated or advisable?"—*Anonymous.*

A. "There are two very good reasons why the sera of donors should be screened for irregular antibodies.

The first is loyalty to the donor. If he or she has an immune antibody which could cause a transfusion reaction or hemolytic disease of the newborn, the donor should have a blood group card with this information. This is then available to the medical attendant for an eventual admission to hospital or in a future pregnancy. This is a loyalty that cannot be omitted with voluntary donors.

The second reason is that from this screening and identification a good supply of diagnostic sera is obtainable on future donations. Amongst our donors we have those who regularly supply us with anti-D (anti-Rh_0), anti-D + C (anti-Rh_0'), anti-E anti-rh″), anti-c (anti-hr′), anti-K (anti-Kell), anti-Le^a, anti-Le^b and anti-P. Recently an excellent anti-k (anti-Cellano) serum was found during routine screening of donors. This is being reported elsewhere (Lang, B. and T. Lodge—Vox Sanguinis, to be published).

The screen test we are at present favoring is a selected pool of not less than six donors, the cells of which are papainized.

Whatever other controversies there may be over transfused antibodies, minor compatibility tests, etc., these two alone have more than justified the screening of donor sera in the last fifteen years."—*I. Dunsford, Ph.D.*

Q. "For what reasons may one get variations in hemolysin titer from the same donor at different donations, in addition to variations in cells used for hemolysin tests?" — *M. T. (ASCP), Youngstown, Ohio.*

A. "Before assuming that the variations in question are due to changes in the amount of isohemolytic antibody, all sources of technical error must be excluded. Volumetric measurements must be accurate and incubation time and temperature must be standardized. The red cell should be freshly collected from the same donor and washed to remove traces of anticoagulant. The concentration of cells used in the test must be uniform and should always be checked by hemoglobinometry.

One of the most critical parameters, and at the same time the most difficult to control, is the amount of complement. For quantitative

work no reliance can be placed on the complement-level of the donor serum: an independent complement source must be used with inactivation of the donor serum. Guinea pig serum as a source of complement may cause difficulties due to species agglutinins. Fresh, pooled human serum is to be preferred but this must be carefully standardized and preserved by freezing or lyophilization. A known isohemolytic serum should be included in each batch of tests.

Providing one is satisfied that the above parameters are completely controlled, differences of two or more tubes in the hemolysin titer may be considered significant. Little information is available on the range of variation of iso-hemolysin to be expected in normal individuals. If it is accepted that iso-hemolysin is an index of immunity to A or B substances significant changes in the titer of one individual must be interpreted as a response to periodic exposure to these antigens. The blood group substances, or substances closely resembling them, are widely distributed in nature and it has been shown that immunity will develop if these substances are taken orally as part of certain foods."—*A. Richardson Jones.*

Erythroblastosis

Q. "In the past week we have had two newborns who had 4 plus Coombs but which were typed as negative by the slide method which we use routinely. The first one is a group O and the second was an A with an AB negative mother. We could not reconcile the developing jaundice, the 4 plus Coombs and the negative typing. We therefore did a tube Rh using some tube typing serum which we have available for D^u detection and obtained a strong positive in each case.

This poses a problem for me in trying to establish a procedural routine. These experiences lessen our faith in the slide method of typing. I should like to know what you would suggest as indications for performing the tube method so that I can work into the routine for the technologists. Do you produce a typing serum for use with the tube method?"—*M.D., Waukesha, Wisconsin.*

A. "We strongly urge that you do not stress the Rh status of infants at birth. The important question is, does the infant have erythroblastosis fetalis or not? The diagnosis is established by the Coombs test. The decision and management depends on maternal history, antenatal titers, the clinical manifestations of the disease in the infant and the laboratory findings in the cord blood which include hemoglobin, hematocrit, reticulocytes, nucleated red blood cells, bilirubin level. If it is decided that transfusion is necessary it is important that the blood used be compatible with that of the mother. The determination of the mother's and the infant's ABO groups are of course important and must be taken into consideration in selecting blood for transfusion. The Rh type of the infant at this point is of no importance and in fact too much concern about it may lead to disastrous errors. For example, in the confusion of emergency Rh negative blood may be selected in an unknown case when the problem is truly due to an anti-c (anti-hr′) antibody.

What you say about the problem in determining the Rh type of cord blood which is strongly sensitized, especially with anti-D, is true and I have occasionally seen an infant get into serious trouble because a laboratory reported it to be Rh negative and the case was not handled promptly as a result. In every instance the Coombs test has been strongly positive. I don't know how to Rh type such red cells with confidence and I don't believe that it would be of great value for the immediate clinical situation as I have pointed out above. The fact that one anti-D serum gave you a positive reaction when another did not is not surprising. I suspect that the reaction which you obtained could at least in part be falsely positive because it is possible to get 'positive' tests in strongly coated cells by merely adding albumin solutions of the proper concentration. Such reactions can be obtained either in the slide or in the tube test, the tube test being a little more sensitive because the application of centrifugation gives physical assistance to the immunologic reaction. I do not believe that one can condemn one test *vs.* another on the basis of the experience which you have had. Any slide testing serum can be applied in a modified tube technic, the type of antibodies in this variety of antiserum being such that they will act in albumin whether used with a 40-50 per cent cell suspension on the slide or 2-5 per cent cell suspension in the tube. The use of saline tube testing sera for this purpose of course is another matter."—*T. J. Greenwalt, M.D.*

Fractional Units

Q. "1. The Second Edition of the *Standards for a Blood Transfusion Service* states that 'removal of plasma is carried out within six days of collection' when preparing packed red cells, whereas the 1960 Edition of the *Technical Methods and Procedures* of the American Asso-

ciation of Blood Banks states 'removal of plasma is carried out within 48 hours of collection.' Which of these time periods do you advise we follow?

2. If a rigid container is entered, using the system outlined in the *Technical Methods and Procedures*, for the removal of either plasma or whole blood, can the removed blood or the residual blood in the container be used for transfusion after 24 hours?" – *M.D., Orange, California.*

A. "1. In the preparation of packed red cells, plasma may be removed within six days of collection rather than within 48 hours. Actually, if the packed cells are to be transfused immediately after preparation, the plasma may be removed at any time within the dating period of the blood.

If, however, one wishes to prepare packed cells many days before they are transfused, then it would be necessary to utilize a double bag unit since this will allow for absolute sterility and a standard dating period of 21 days after plasma removal. This would not be permissible with either a rigid or single bag container that would require opening, entering, or venting.

2. If a rigid container is entered for the removal of either plasma or whole blood, the removed blood or the residual blood in the container is safely transfusible for 24 hours only after such removal if the separation has been performed with strictly aseptic technic, and if the blood is continuously held at proper storage temperatures."—*Mark Falcon Lesses, M.D.*

Correspondence

January 27, 1961

To the Editor: "Rare Blood and International Registration."

"The discovery of new blood factors, their distribution in different races and their heredity is becoming more and more a great center of interest. I myself was very much interested in the letter of 'to the Editor' entitled 'Consanguinity and certain rare blood groups' in *Vox Sanguinis*, 5: 383-384, 1960 which was written by Race and Sanger.

In 1957, I found and reported the first case of $\overline{\overline{R}}^{o}$ (D– –) in a Japanese patient. I forwarded my findings to Dr. Philip Levine, Ortho Research Foundation, Raritan, New Jersey, U.S.A., who confirmed this unusual blood and reported it at the AABB meeting in Chicago in 1957. Publication of this first case appeared in the Proceeding of the Japan Academy, 33 (4): 233-235, 1957 and the Ochanomizu *Medical Journal*, 6 (2): 105-137, 1958, which is a monthly issue from the Tokyo Medical and Dental University. However, the results of my investigation of $\overline{\overline{R}}^{o}$ (D– –) blood were not completed and unsatisfactory because of the presence of some unusual phenomenon of antibodies in the serum of the propositus at that time.

Soon after that in 1958, Dr. Masayori Kuniyuki, NICHIYAKU Blood Bank, Tokyo, Japan, found another family of $\overline{\overline{R}}^{o}$ (D– –) in Japanese and the serum was tested with $\overline{\overline{R}}^{o}$ (D– –) blood which was previously discovered by me. The patient's serum did not agglutinate the $\overline{\overline{R}}^{o}$ (D– –) cells but more than 200 random samples were agglutinated by this serum. We again asked Dr. Philip Levine for confirmation of this blood and he identified it. Further investigation of these two Japanese families of $\overline{\overline{R}}^{o}$ (D– –) has been done recently and we are expecting to send the manuscript to your journal of 'TRANSFUSION' as soon as it is completed.

Since then another case of $\overline{\overline{R}}^{o}$ (D– –) blood has been found in Japanese and identified by Col. Joseph H. Akeroyd, Department of Immunohematology, Walter Reed Army Institute for Research, Washington, D. C., U.S.A.

Surprisingly enough, the fourth case of $\overline{\overline{R}}^{o}$ (D– –) blood was found very recently by Dr. Taro Tsuchiya, Hiroshima Blood Bank. I worked with Dr. Tsuchiya and his blood research group on this sample. The serum brought to notice the presence of unusual antibody therefore, we asked Dr. Ivor Dunsford, National Blood Transfusion Service, Regional Transfusion Centre, Sheffield, England for further study of this sample.

According to the letter of December 20, 1960 from Dr. Dunsford, the red cells of this patient were again $\overline{\overline{R}}^{o}$ (D– –). We are still investigating this blood and family at the present time.

These newly discovered cases in Japan have raised world wide interest and helps to make this a global problem in the transfusion field. I am hoping that with your active help the storage of such rare blood for emergency transfusions can be brought about easily and quickly.

At first, we thought these $\overline{\overline{R}}^{o}$ (D– –) types in Japanese were isolated people who have been living in a small village in Japan and were the result of consanguinity due to intermarriage. We are going to contact these families and re-

quest permission to place their names on an international register for purposes of emergency transfusions and hope that all families, regardless of location, in the world will join us in this movement." — Sincerely yours, *Mitsuo Yokoyama, M.D.*

February 7, 1961

To the Editor: "Many thanks for giving me the opportunity to reply to the letter from Dr. Yokoyama in regard to 'Rare Blood and International Registration.'

The Committee on Reference Laboratories Program of the American Association of Blood Banks has for several years been concerned with the problem which Dr. Yokoyama mentions in his letter.

In order to meet the need for transfusion therapy in patients who presented difficult serological problems the following steps have been taken so far:

1. Establishment of four Central and 15 Regional AABB Reference Laboratories. The function of these reference laboratories is two-fold:
   a. to service blood bank laboratories in all cases where they need help with laboratory problems, in particular with identification of blood group antibodies and special rare types of blood.
   b. to help blood banks locate compatible donors, in particular for cases of sensitization to blood group antigens which are present in the blood of the vast majority of donors.
2. Establishment of a Central File of rare donors.
   This file, which is located in the Milwaukee Blood Center has, during approximately two years, compiled an extensive list of 1,114 donors of rare and rarer types. This list has been compiled by the coordinated effort of 46 laboratories. It contains a substantial number of very rare donors, such as k (Cellano) negative, Tj^a-negative, U-negative, Lu^b-negative, Vel-negative, D— — and other similarly rare types. The central file has on several occasions been of great value in obtaining blood for patients of rare types.
3. Establishment of a cooperative program by the AABB Reference Laboratories Program in cooperation with the Blood Research Laboratory, Chelsea Naval Hospital, Chelsea, Massachusetts and the Clinical Center Blood Bank Unit, Laboratory of Blood and Blood Products, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland. Under this program units of blood of extremely rare types are being stockpiled frozen in glycerol for future need. Thirty-five units of extremely rare bloods, such as k-negative, U-negative, 'Bombay' and others are already in frozen storage and it is hoped that the number will increase rapidly in the near future.
   The Reference Laboratories Program of the American Association of Blood Banks has extended invitations to laboratories in England, Holland and other countries to participate in this program and it is indeed a pleasure to take this opportunity to extend an invitation to our friends in the Blood Transfusion Services in Japan and other countries to join us in our efforts to build up a sufficient list of rare donors and a stockpile of extremely rare bloods.

It is hoped that each country will organize its own central file for rare donors and that worldwide communication be established between these files for the benefit of patients with extremely rare blood types.

It is also hoped that many additional depots for frozen rare type bloods can be established in the U.S.A. and throughout the rest of the world." Sincerely yours, *Morten Grove-Rasmussen, M.D., Chairman, American Association of Blood Banks Committee on Reference Laboratories.*

December 23, 1960

To the Editor: "It was very encouraging to note in the 'Report of Activities of the Central File for Rare Donors' in the November issue of the Bulletin of the American Association of Blood Banks, that while the registered donors are listed in terms of the C-D-E notations, *e.g.*, CDe/C, cde, cD^we, CDE/C^we, and the like, the requests received for blood were mostly submitted in the standard Rh-Hr nomenclature, *e.g.*, Rh_2Rh_2, Rh_1Rh_1, rh′rh′, etc. This demonstrates once more that properly informed intelligent persons prefer what is correct and precise —no matter how complex—to what is inexact or fallacious, even when the latter is superficially attractive and simple. The reason is that in the long run the truth, no matter how complicated, leads to the correct answers, and thus simplifies matters, while fallacious terminology and reasoning result in blunders which if not fatal are certainly embarrassing." (Wiener, A. S.: Modern Blood Group Mythology. Jour. Forensic Med., **7**: 166-176 [Oct.-Dec.], 1960.)— *A. S. Wiener, M.D.*

Either nomenclature is acceptable for publication in *Transfusion.—Editor.*